Minireview

From entangled membranes to eclectic morphologies: cubic membranes as subcellular space organizers

Tomas Landh*

Department of Biophysical Sciences and Biomaterials, State University of New York at Buffalo, Buffalo, NY 14214, USA

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Abstract The identification of evolutionary conserved membrane morphologies whose architecture is governed by cubic symmetry – cubic membranes – adds a new dimension to cell membrane functions and, perspicuously, to their role in subcellular space organization. Through analysis of electron micrographs, three families of cubic membranes have been unequivocally identified in which one or more (parallel) membranes, described by periodic cubic surfaces, partition space into two or more independent, albeit convoluted, subspaces of membrane potential determined dimensions. The choice of a particular cubic symmetry is suggested to be due to its activity. Here the architecture and function of multiple (≥ 3) subspace organization in classical membrane bound organelles is addressed. As it can be precisely determined with cubic membranes suggests that they can be employed as a reference morphology.

Key words: Transmission electron microscopy; Image analysis; Cell space organization; Cell membrane; Cubic membrane

1. Introduction

Traditionally, cell membranes are pictured locally by means of the fluid mosaic model. Their global organization, i.e. morphology, ranges from highly organized, such as tight junctions, to irregular assemblies, perhaps best exemplified by the classical view of smooth endoplasmic reticulum (SER). Furthermore, membrane bound cell space is divided into organelles depending on their primary function and apparent biochemistry. In this scheme of thinking, each organelle is thought to be bound, and its morphology shaped, by the membrane(s) enclosing its active space(s). However, even though a shape tells us something about the forces that molded it, this model leaves the three-dimensional (3D) topology and continuity of the spaces embedded in the particular organelle open to interpretation. One reason for this ambiguity is that we are to a large extent limited in our study of cell membrane-based morphologies and, in particular, their spaces to the use of transmission electron microscopy (TEM) techniques which involve an ambiguous deciphering of the two-dimensionally (2D) projected representation of the membrane(s) and space(s) they enclose. I feel that much of this uncertainty depends on the hitherto lack of an evolutionary conserved 'reference' membrane morphology whose 3D structure is exactly known and theoretically predictable. This problem was recently overcome by the discovery of the ubiquity of cell membrane morphologies with cubic symmetries [1]. These are unambiguously described by so called triply periodic cubic surfaces (PCS), of which two mathematical representations, the periodic minimal and periodic nodal surfaces, are basically geometrically indistinguishable [1]. Without understanding of their 3D structure, these membrane morphologies have been reported in the literature in thousands of TEM studies for more than 35 years and given such nicknames as 'undulating membranes', 'paracrystalline membranes', 'cotte de maille', 'lattice organelle', and 'tubuloreticular structures', to mention a few. They are now naturally termed *cubic membranes* [1,2].

Cubic membranes are extraordinary efficient space organizers in which one or more continuous intersection-free membranes, each draped over a PCS, divide space into two or more physically distinct subspaces, each topologically compatible with the barrier function of cell membranes. In view of this capacity of cubic membranes, which has been revealed by the analysis of TEM micrographs [3,4], some concerns regarding the traditional view of cell membrane partitioned spaces are addressed, and a more optimal solution to the general problem of cell membrane bound space organization in which three and more spaces of organelles can coexist is suggested.

2. Cubic membranes

Through the development of a direct template correlative (DTC) matching method based on pattern and symmetry recognition between the TEM micrographs and theoretical computer generated projections of PCSs [1–4], three fundamental families of cubic membranes have been identified [1]. Namely, the cubic membranes based on the gyroid (G) of Schoen [5], and Schwarz' double diamond (D) and primitive (P) surfaces [6], or their structurally analogous nodal surface representations [7]. The principal members of these families are exemplified in Fig. 1. The conceptually most simple surface arrangement of these is a single continuous membrane that drapes the balanced (zero potential, or analogously zero mean curvature) PCS as illustrated in Fig. 1a–c, displaying a unit cell of the G-, D-, and P-surface, respectively. However, cell membranes also form non-balanced cubic membranes in which the surface potential describing the PCS is non-zero, causing it to be (orthogonally) displaced relative to the zero potential level. In addition to the

*Corresponding author. Fax: (1) (716) 833 3517.

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; DTC, direct template correlation; D, double diamond; G, gyroid; PCS, periodic cubic structures; P, primitive; SER, smooth endoplasmic reticulum; TEM, transmission electron miscroscopy.

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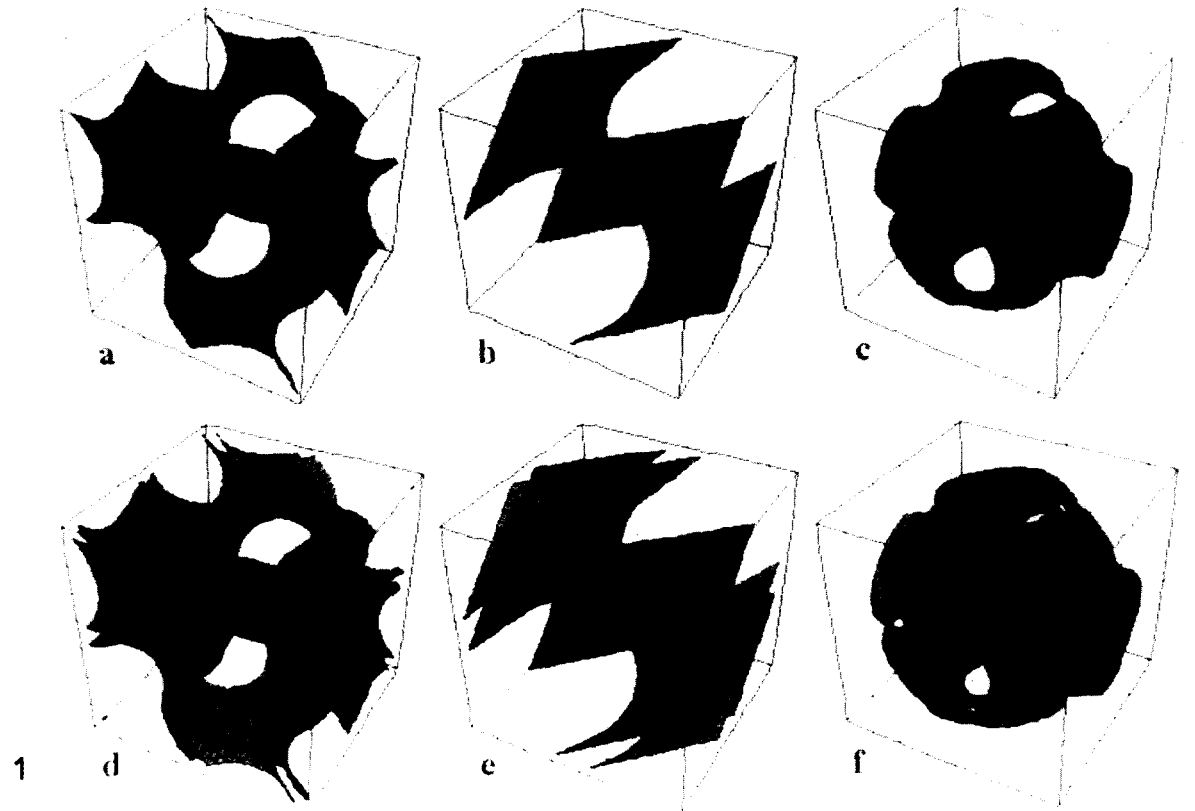

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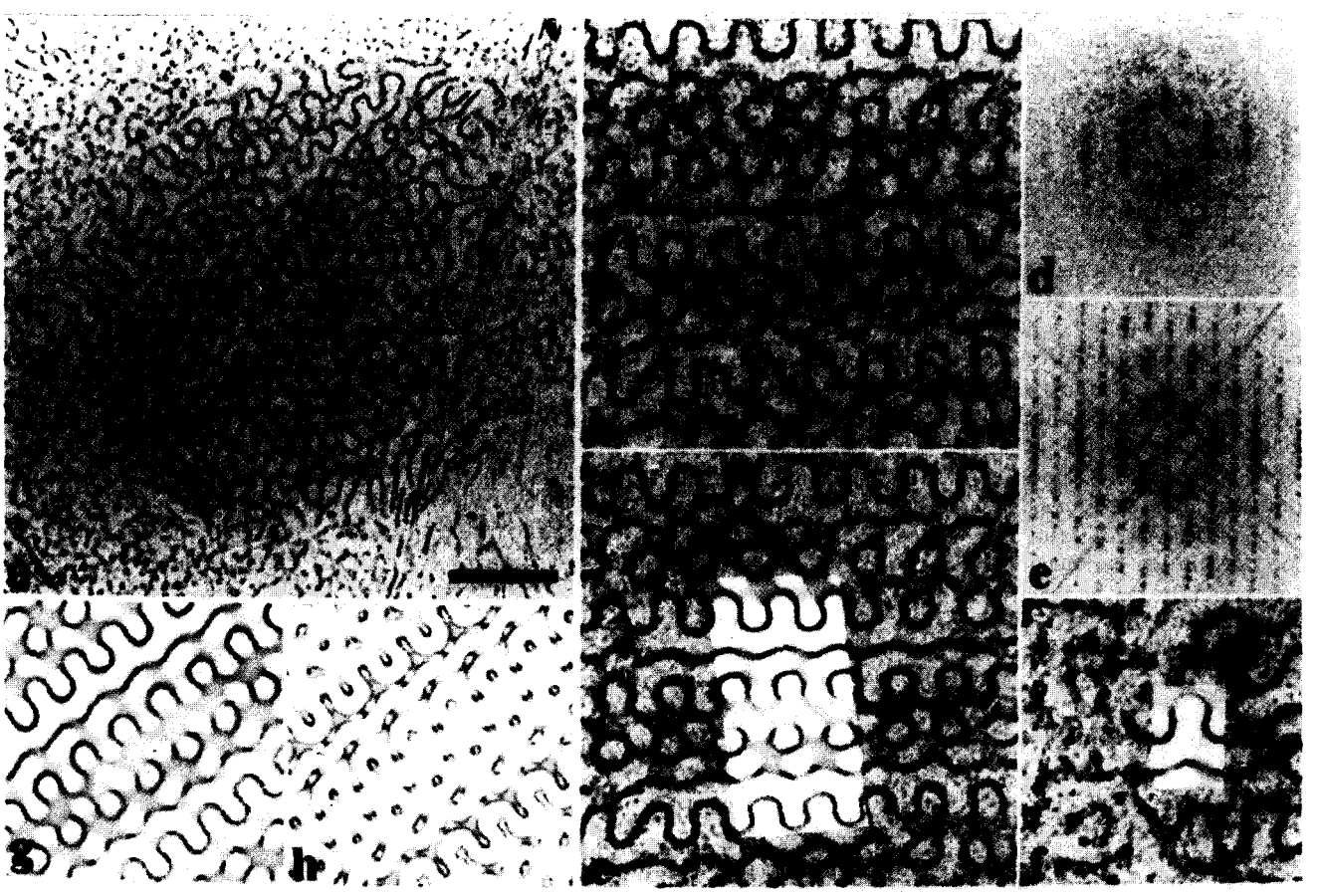

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single membrane case, cell membranes form a hitherto unknown (also to mathematicians) construction of PCSs in which several (independent) parallel membranes are arranged according to their potential, which can be either balanced, as shown in Fig. 1d–f, unbalanced or simply displaced relative to the zeropotential surface. The latter will be referred to as multicontinuous or foliated.

Through the use of the DTC method, thousands of published and unpublished studies which unknowingly deal with cubic membranes have been identified, including representative examples from all major cell types and all kingdoms [1]. They appear in conjunction with virtually all cell membranes, though they are most frequently formed by the SER (including the inner and outer nuclear membrane), inner mitochondria membrane, plasma membrane, and membranes belonging to the endosomal pathway (see [1,3] for comprehensive lists). A practically unambiguous identification of projections of all members of all three families of cubic membranes have been performed in manifold cases reporting various, hitherto perplexing membrane morphologies [1]. These are exemplified by a SER G-type of single balanced cubic membrane [8], an unbalanced plasma G-type membrane [9], a multicontinuous mitochondrial G-type [10], a single balanced D-type in mitochondria [11], the unbalanced D-type of the prolamellar body (PLB) [1], a SER multicontinuous D-type [12], an intranuclear single membrane balanced P-type [13], the unbalanced P-type [14], and, finally, a mitochondrial multicontinuous P-type cubic membrane [15]. The lattice parameter of a cubic membrane seems to be highly controlled, and is often maintained constant within a certain cell type at a given time. Between the cell types, however, it often varies significantly, with both the number and potential of the parallel (foliated) membranes, and can be from 50 nm (e.g. the PLB) to above 500 nm (e.g. the P-PCS based foliated cubic membrane identified [1] in the work by Barber [16]. Nevertheless, in certain cases such as the PLB, the lattice parameter is rather constant (50–60 nm) independent of species investigated so far (Mieczkowski and Landh, unpublished).

Most often cubic membranes seem to occur at specific demands put by the cell machinery during both normal and pathological development or regeneration. In some cellular organelles such as the mitochondria of sertoli cells and the chloroplasts of higher plants, they occur at specific events during normal differentiation and development [1], but they apparently also develop as a specific regenerative or pathological response [1] such as those frequently called tubuloreticular structures (see e.g. [17]).

3. Space organizations in cubic membranes

As depicted in Fig. 1, PCSs partition global space into two not necessarily congruent sub-spaces. In conjunction with cell membranes, these spaces are of course always different, due to the inherent membrane asymmetry, and the space group of a cubic membrane is hence always of the black and white subgroup. Analogously, if a cubic membrane is made up of n parallel membranes, as shown in Fig. 1d–f, the cubic membrane partitions space into $n + 1$ subspaces. The space group is still the same as for a single membrane, but its crystallographic motif is now more complex. The global topology of a cubic membrane depends on the continuity (how its ends are constructed) and the constellation of the membrane(s) constituting it. Since the topology of the PCS describing the cubic membrane is always well defined, one can study the genesis as well as its global topology and continuity in relation to it. The best cases for the study of these topological relationships seem to be the mitochondria, since they represent a (mathematically and physically) closed form of a cubic membrane. An interesting system seem to be various species of the giant amoeba *chaos* [1].

In projections, like those produced in TEM, of membrane based morphologies, information about the relationship between continuity and dimensions of physically independent spaces is rarely unambiguous, even if serial sectioning or other reconstructive techniques are employed. In the case of cubic membranes, the situation is, however, opposite since we recognize it through its morphological and crystallographical complexity rather than reconstructing the 3D structure, and most importantly, through the extreme preservation of the finest details in its projected electron density map. The two first properties have made cubic membranes escape identification for such a long period of time, but they serve as a signature of the particular type of cubic membrane. An illustrative example of this pattern and recognition procedure applied to a 'well ordered network of SER' [18] is given in Fig. 2. Once the type of cubic membrane and the projection direction is recognized, the specimen thickness and membrane potential account for the fine variations in the electron density map, which makes it possible to apply the (known) space relation to the TEM micrograph. In the multicontinuous cubic membranes where more than two spaces coexist, this can be done by assigning each side (half bilayer thickness) of the membranes facing the same space, (the same colour as in Fig. 1d–f) and comparing it to the matched gray scale PCS projection (Fig. 2e and g). This proce-

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Fig. 1. The periodic cubic surfaces describing the three fundamental cubic membranes. (a)–(c) Balanced, zeropotential G-, D- and P-PCSs, respectively, in which the PCS partition space into two distinct subspaces of equal dimensions embraced by either the red or blue side of the PCS. (d)–(f) Multicontinuous constellations of the G-, D- and P-PCSs, respectively, each exemplified by two equipotential (±0.25) PCSs, i.e. balanced around the zero potential levels.

Fig. 2. Multiple space relationships in cubic membranes. (a) Micrograph of SER in Müller cells of the pika retina showing two distinct morphological appearances – double membrane G-PCS based morphologies, with different membrane potentials (Bar, 1 μm). (c) DTC analysis of an enlarged portion (b) revealing it to be a projection of an approximately 75 nm thick section along the [853] direction of a double G-type, in which the two membranes have potentials of about ± 0.20 – the theoretical projection (g) matched in (c). Note that in the first large template (corresponding to the projection of 25 × 1/4 of unit cells) random noise has been introduced. The second match, with coloring of the spacial sidedness of the membranes, is fit without such adjustment (its upper and lower bounds are the same as the first template). The projective organization of the three spaces (red, blue, and green sides of the membranes) would be practically impossible to deduce without the aid of colour assignment. (d) and (e) are the FFTs of (b), and the undistorted corresponding [853] G-PCS based projection, respectively. The seemingly non-symmetrical morphology corresponds to a gyroid cubic membrane in which the membrane potentials are approximately ± 1.25. Such high potential PCS constellations give rise to two interpenetrating tubular-like membrane bound spaces (blue and green in the [853] projection shown in (h) (with color assignment corresponding to that in Fig. 1d–f) by which their relationship to can be shown to be the same as that in the ± 0.2 constellation (f). Original TEM micrograph courtesy of Hitachi, Ltd. [18].

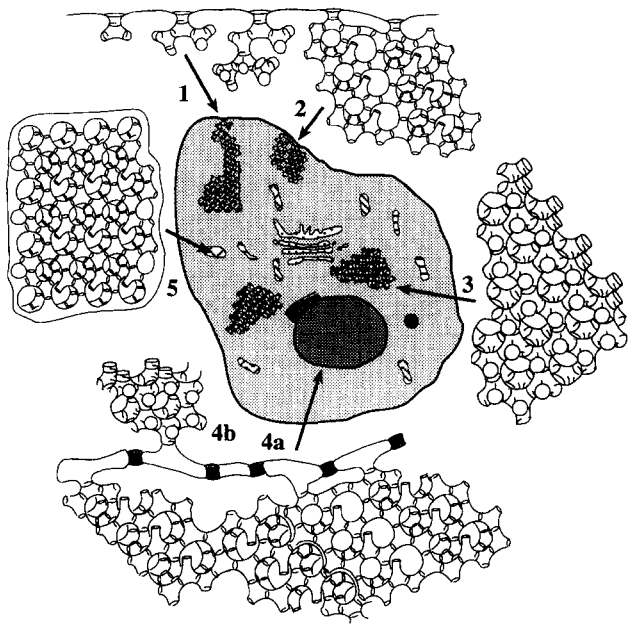

Fig. 3. The most frequent sites of identification of cubic membranes in mammalian cells. (1) Infoldings of the plasma membranes shown to occur along a common 'flow-growth' process, best examplified by the endosomal (non-clathrin mediated) pathway along which a continuos lattice transformation can take place. (2) Non-endosomal membrane infoldings leading to the formation of a cubic membrane examplified by a nonbalanced single G-type membrane whose topology depends upon the number of invaginative pores. (3) Cubic membrane of the SER, which is the most frequently, together with mitochondria, identified site. In only a few cell types have we identified lattice transformations, or the coexistence of different cubic membranes. Shown is a non-balanced single D-type membrane. (4) Membrane foldings forming cubic membranes of the outer (a), i.e. SER, and inner (b) nuclear membrane examplified by a non-balanced G-type (a) and D-type (b). It has been shown [1] that foldings along (a) often lead to the formation of multiple (≥ 3) spaces of the SER. There are many (topological) ways to form these multimembranous cubic membranes, examplified here by the interpenetration of two (spatially independent) cubic membranes. (5) Mitochondrial cubic membrane. Both single and multicontinuous cubic membranes have been identified [1]. Shown is a non-balanced single G-type membrane.

dure allows us not only to directly deduce space relations, but also to trace the meandering paths they take [4]. Once the lattice parameter and membrane potentials are deduced, the continuity, surface area as well as number, dimensions, and relations of intracellular membrane bound spaces cannot only be rigoursly studied and measured in the cubic membranes, but, I think, should be used to reconsider cell space organization. This is particularly true in light of the apparent existence of multiple intraorganellar spaces [1,4]. Recently, we have applied this to morphological transformations, i.e. to continuous membrane folding processes [1] (see Fig. 3) between cubic membranes and non-cubic structures, such as simple lamellar close packing of membranes, in order to trace the relationship of the spaces, which are otherwise indistinguishable in these morphologies [4], and have shown that they are maintained. Thus, even in what appears as simple morphologies, the space organization can be multifarious.

4. Eclectic order versus entangled disorder

Since cubic membranes apparently are extraordinarely well preserved in TEM experiments, it is most important to compare them to the classical view of cell membrane morphologies and, in particular, to find any commonalities between them. The morphology of the SER in Fig. 2 will serve as an example. The definition of morphological order and disorder, will be that of symmetry and the apparent lack of it, respectively.

The most frequent 3D model of SER consists of continuous meandering tubules with undefined topology and symmetry such as seen in the periphery of Fig. 2a. The models, however, usually involve one and only one (continuous) membrane. Thus it can alternatively be viewed as one single membrane which separates space into two subspaces, very similarly to a cubic membrane. The major difference is the lack of symmetry. As a first attempt to compare the ordered vs. disordered morphologies, we might search for a continuous (constant topology, i.e. continuous membrane) perturbation of a cubic membrane which gives rise to the classical tubular-like SER morphology. Indeed, the larger the potential, i.e. the larger the subspace volume difference(s) are in a cubic membrane, the more tubular-like it appears. The tubular-like morphology in Fig. 2f and h is such a cubic membrane. However, it is made up by two parallel membranes maintaining the three spaces! Since the surface potential is greater than one (approximately $\pm$ 1.25), the transformation from the G-PCS based $\pm$ 0.2 morphology is, however, not continuous. We now understand that such unfolding or regular morphological transformation of cubic membranes often causes the overall morphology to appear as that of the traditional view of SER. More importantly, if it is assumed that the transformation is continuous, the topology is constant, and the same space partitioning as in the cubic membrane is maintained. Of particular interest is the gyroid morphology depictured in Fig. 2 since it has a unique behavior at the critical absolute membrane potential of one, where the continuity of the membrane(s) is usually broken. The so called t-tubule of skeletal muscle, which invariantly forms a G-PCS based cubic membrane with a varying potential (Whitford, W. and Landh, T., unpublished), seems to be particularly well suited for studying this transformation.

5. Functional aspects of cubic membranes

As we are beginning to understand some aspects of the morphogenesis of cubic membranes, it is important to point out some of their basic properties. Though their crystalline-like appearance, cubic membranes are not crystals in the true sence, and hence share only the principal geometrical membrane arrangement with the condensed cubic phases [1]. This only considers the difference between phase space and cell space, and one can argue that the periodic minimal representation is a valid description of cubic phases in the former, and the periodic nodal representation of the cubic membranes in the latter. Cubic membranes are thus, rather, dynamic crystals of fluid films, were 'dynamic' emphasizes the continuous changing composition within the apparent constant crystallographic arrangement. How this is maintained remains an enigma. It may, however, be due to an overall maintenance of chemical potentials driving certain reactions or membrane flow with a high demand perhaps at one the time.

Besides their, perhaps, primary function as a space organizers, the symmetry of cubic membranes can also be used for specific activities, and the fact that some of cubic membranes are structurally invariant in different species implies such a radical suggestion. Namely, that the specific *symmetry*, i.e. the particular underlaying PCS is chosen in order to fulfill a function. Such a choice would be based on a purely structure-functional relationship depending upon the symmetry alone, and it would add a hitherto unaccounted for functional arena of cell membranes [1,2,19]. The mechanism for such a choice is, however, most obscure. But it apparently must exist, since cell organelles whose function is similar, e.g., the mitochondria in certain species of *Chaos*, form P-type multicontinuous cubic membranes of virtually constant lattice size and defining three spaces during a certain time period of the life cycle. Since the membrane composition, mitochondria size, and external morphology vary, there are most certainly other pressures to form these three spaces embraced only in the P-type, and not in the G-, or D-type of cubic membrane.

The best developed theoretical account for a specific structure-functional relationship is the yet to be experimentally proven existence of a photon band gap in the structure of the prolamellar body in etiolated and developing higher plants which would trap photons of suitable wavelength to be used in the conversion of protochlorophyll to chlorophyll [2,3,19].

6. Accounting for cubic membranes

A unique feature of the cubic membranes is the fact that as shown above they allow us to discriminate the 3D spatial relationships through their 2D projections. The simplest discretization (when only one membrane is involved) is best exemplified by plasma membrane invaginations, several different types of which give rise to cubic membranes as schematically illustrated in Fig. 3. Also, the cubic membranes identified in SER and mitochondria frequently form these single membrane types in keeping with the traditional number of partitioned spaces in these organelles (Fig. 3). However, the multicontinuous cubic membranes are commonly identified in these organelles, and the biogenesis of the spaces embraced in them is yet to be elucidated. The intriguing idea that there are several independent spaces within certain organelles deserves, however, serious thought – particularly if these spaces indeed carry distinct, yet related, functions. Since the classical SER is formed as continuous foldings of the nuclear envelope, the simplest topological formation of multiple SER spaces seems to be through multiple sets of its foldings as illustrated in Fig. 3. There are, however, numerous other, though more complicated, foldings that can give rise to such multicontinuous cubic membranes, some of which account for the formation of the cubic membranes in mitochondria.

Though I have sofar only addressed cubic PCS architecture of cell membranes, it is most important to point out that there are other higher symmetries governing cell membrane morphologies. Particularly, various periodic hexagonal and tetragonal surfaces seem to describe a manifold of morphologies such as the so-called annulate lamellae (Landh, unpublished). Thus, it seems to me that in all such membrane morphologies, in which symmetry is governing the principal architecture – so beautifully manifested in the cubic membranes – mathematics, physics, molecular and cell biology may begin to merge.

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