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What history tells us XXX. The emergence of the fluid mosaic model of membranes

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1. Introduction

In 1968, Walther Stoeckenius summarized in *Science* the conclusions of a meeting held the previous year at Frascati near Rome on 'membrane modelling and membrane formation' (Stoeckenius 1968). The diversity of the issues that were raised, and of the methods and models that were used, was such that no consensus could be reached. At that time, even the existence of a cell membrane was not unanimously accepted! Three years later, S Jonathan Singer (1971) proposed the mosaic model of the structure of cell membranes to replace the previously dominant Danielli–Robertson unit membrane model, and one year later he and Garth L Nicolson added the word 'fluid' to 'mosaic' (Singer and Nicolson 1972). The new model was rapidly and unanimously accepted, and it remained unaltered during the next forty years.

This rapid evolution of models of the membrane structure has not been explored by historians. I will show that the emergence of the fluid mosaic model was not as smooth as indicated in the description given by one of its authors (Singer 2004). What happened in the mid-1960s was an unsuccessful attempt to replace the lipid bilayer model, also more precisely named the 'bimolecular lipid leaflet' model, by a new model giving proteins a major role in the organization of membranes. The unexpected resistance of the lipid bilayer model, as well as new data demonstrating the rapid displacement of proteins within the membrane plane, led to a progressively elaborated synthesis that was brilliantly outlined by Singer and Nicolson in their famous *Science* article.

2. The challenges to the lipid bilayer model of membrane structure in the 1960s

Careful measurement of the amount of lipids present in the membranes of red blood cells led E Gorter and F Grendel to propose in 1925 the existence of a bimolecular layer of lipids in cell membranes (Gorter and Grendel 1925). Ten years later, Danielli and Davson generalized the previous observations (Danielli and Davson 1935). In the 1950s Robertson made slight modifications to the model to make it compatible with electron micrographs (Robertson 1959), showing that the membranes were formed of two dark bands of 20 Å separated by a clearer one of 30 Å: he added on both sides of the lipid bilayer two thin layers of extended proteins interacting with the polar heads of lipids. The model was renamed the unit-membrane model (for a more detailed history of these early models, see Robertson 1981).

It was criticized in the 1960s. I will not provide the details of the numerous studies that prompted these criticisms, but only sketch the main theoretical and experimental approaches that challenged the unit-membrane model. A full description can be found in the reviews written during these years (see, for instance, Korn 1966, 1968, 1969).

A general criticism was that the so-called 'paucimolecular' unit-membrane model was unable to account for the functional and structural diversity of membranes. Too many studies had been focused on myelin, which, although derived from the membrane of Schwann cells, has a unique function to fulfil – insulation of the nerve fibre.

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Electron microscopy findings supporting the unit-membrane model were contested. Not only might the fixation and staining procedures alter the samples, but the interpretation of the micrographs was problematic because it was not founded on serious chemical data (Korn 1966).

Observations on membranes from chloroplasts and mitochondria had revealed the existence in these membranes of 'subunits' or 'particles' (see, for instance, Parsons 1963). The existence of 'subunits' was rapidly demonstrated in other membranes: bacterial membranes (Razin *et al.* 1965) and outer segment membranes from retina (Blasie *et al.* 1965). The new freeze-etching technique of electron microscopy developed by Daniel Branton (1966) circumvented staining artefacts and also revealed the existence of particles within membranes.

Thermodynamic considerations and physicochemical measurements also shook the unit-membrane model. Singer underlined the importance of hydrophobic interactions between lipids and proteins in the stabilization of the membrane structure, as well as the poor thermodynamic stability of the structure represented in the unit-membrane model.

Direct measurements by optical rotatory dispersion and circular dichroism of the structure of the proteins present in the membranes of red blood cells and of *B. subtilis* bacteria demonstrated the abundance of α-helical structures hardly compatible with the unit-membrane model (Lenard and Singer 1966). The occurrence of a red shift in the spectrum was interpreted as the result of interactions between membrane proteins (Lenard and Singer 1966; Steim and Fleischer 1967).

3. The emergence of new models of membrane structure

Alternative models of membrane structure were proposed (Benson 1964; Green and Perdue 1966; Lenard and Singer 1966). There was no agreement on a possible classification of these models (Rothfield and Finkelstein 1968; Stoeckenius and Engelman 1969). I will adopt the point of view of Stoeckenius, who gathered these different models under the banner of 'subunit models'.

The hypothesis that the diversity of membrane structures might be generated by a diversity of lipid structures remained marginal (Luzzati and Husson 1962). It was unanimously considered that the main components in membrane organization were proteins, which could be rather independent lipoproteins or structural proteins (Richardson *et al.* 1963). The relative positions of protein and lipids as well as the orientation of the lipid molecules differed from one model to another and were the object of debate. The diversity of the subunits present in the membranes was also a matter of debate. But, what was common between these different models was the conviction that proteins interacted as in a crystal (Vanderkooi and Green 1970) to create the architecture of membranes. This conviction was supported by experiments showing that lipid depletion (Fleischer *et al.* 1967; Napolitano *et al.* 1967) and phospholipase C treatment (Lenard and Singer 1968) did not alter the structure of membranes.

This dominant position of proteins in the structural organization of membranes (as opposed to the previous vision) was the result of the recent developments of molecular biology. When Jean-Pierre Changeux proposed to extend the allosteric model of protein transconformation to account for the phenomena of cooperativity observed in membranes, he considered as a well-established fact that 'membranes are made up by the association of repeating globular lipoprotein units' (Changeux *et al.* 1967, 335). For molecular biologists, proteins are in charge of cellular functions and, as expressed by Korn, 'form follows function, rather than function being restricted within the confines of structural limitations' (Korn 1966 p 1497). The macroscopic morphology had to emerge from the self-assembly of protein components (Monod 1972). Evidence for such a process accumulated at the same time for phages and viruses (Edgar and Wood 1966; Finch and Bancroft 1968). Simple physical principles guided the construction of regular structures from subunits (Caspar and Klug 1962).

Therefore, between 1966 and 1972, there coexisted different models of membranes that shared the same principle: the structure of membranes resulted from the structure of their proteins and from the interactions of these proteins. The construction of the fluid mosaic model was not an extension of these early models, but the result of a 'synthesis' demanded by the resistance of the lipid bilayer model of membranes, which was unexpected by molecular biologists.

4. The resistance of the lipid bilayer model, and the construction of the fluid mosaic model

The most obvious sign of resistance of the lipid bilayer model came from freeze-etching electron microscopy. This technique unambiguously demonstrated the presence of globular proteins within membranes. But it also confirmed the initial intuition of Branton that the fractures observed on the pictures occurred between the two leaflets of lipids. It demonstrated the existence of these two leaflets, and their major role in the structural organization of the membranes (Deamer and Branton 1967; Pinto da Silva and Branton 1970).

The production of artificial membranes by Mueller in 1962 and of vesicles (liposomes) from lipids demonstrated that the lipid bilayer model retained its explanatory value (Sessa and Weissmann 1968; Henn and Thompson 1969), not only because of the predominance of the bilayer structure in these artificial membranes, but more significantly because these artificial membranes mimicked most properties of biological membranes (Rothfield and Finkelstein 1968; Henn

and Thompson 1969; Steim *et al.* 1969) – but not all, for instance not the permeability to ions. One interesting example of this similarity was provided by Albert Lehninger. Uncoupling agents such as 2-4-dinitrophenol had been described by researchers studying oxidative phosphorylation in mitochondria. Within the framework of the chemiosmotic coupling model that he had proposed, Peter Mitchell hypothesized that these molecules were able to cross the membranes and to abolish the proton gradient between the two faces of the inner mitochondrial membranes (Morange 2007). Albert Lehninger and his coworkers showed that uncoupling agents increased the permeability to protons of artificial membranes (Hopfer *et al.* 1968). This result not only provided strong support for the new chemiosmotic theory, but also demonstrated that the barrier to permeability characteristic of biological membranes was due to the existence of a lipid bilayer.

The last step towards the fluid mosaic model was the demonstration that diffusion within the plane of biological membranes was free and rapid. The first evidence came from the results obtained with spin labels attached to lipid molecules (Hubbell and McConnell 1969). A careful study of the observations made on the retinal rod outer segment membranes also led Vanderkooi and Sundaralingam (1970) to propose that proteins float freely in the lipid bilayer.

The experiment that struck the minds of biologists, and convinced them of the existence of a rapid lateral diffusion within membranes, was performed by Larry Frye and Michael Edidin in 1970 (Frye and Edidin 1970). Using the new technology of cell fusion developed by Henry Harris (1995), they fused mouse and human cells and showed by indirect immunofluorescence that the antigens present at the surface of the two cells were rapidly mixed in the membrane of the heterokaryon. The experiment was not entirely new – similar results had been obtained three years before by Watkins and Grace (1967), but the quality of the images obtained by Frye and Edidin, their resolution in time, as well as the abundance of controls that they performed to eliminate other possible explanations for the rapid intermixing of antigens gave their experiment a huge impact. One year later, Martin Raff and his colleagues described the rapid redistribution of immunoglobulins at the surface of lymphocytes B after the addition of an anti-immunoglobulin antibody, a phenomenon called 'capping' (Taylor *et al.* 1971). Whatever its physiological significance, capping confirmed the rapid diffusion of proteins within the plane of the membranes. This observation was compatible with the lipid bilayer model of the membrane, but incompatible with models proposing that membranes were organized in a quasi-crystalline state by interactions between their protein constituents.

The issue was no longer to replace the lipid bilayer model by a new model putting proteins at the heart of membrane organization, but to accommodate in a synthetic model the main characteristics of membranes that had been confirmed or had emerged in recent years: the central place of the lipid bilayer, the asymmetry of the membrane, the insertion of proteins within the membranes (and some spanning the membrane), the distinction between these integral membrane proteins and proteins that are only loosely attached to the membranes, and the importance for the stabilization of membrane structure of the hydrophobic interactions between the apolar amino acids of these integral membrane proteins and the apolar part of the lipids. None of these characteristics of the fluid mosaic model was new: the existence of proteins spanning the membrane in an asymmetric way was elegantly demonstrated by Marc Bretscher (Bretscher 1971, 1972), but it had been proposed years before that α-helices were able to span the thickness of the membrane (Hoelzl Wallach and Zahler 1966). The relative independence of the lipid and protein moieties in the membrane had also been anticipated because of their different turnovers (Omura *et al.* 1967). In the latter article, the word 'mosaic' was already used to designate this heterogeneity of membrane domains, but it was not the first introduction of this term to describe the structure of membranes (see, for instance, Benson 1964). The new synthetic vision was the only one compatible with the diversity of structures and functions of biological membranes (Korn 1969).

The volume published by the New York Academy of Sciences in 1972 was a step towards this synthesis (Green 1972). But it was the merit of Singer and Nicolson to present the new model in a simple and attractive way (Singer and Nicolson 1972).

5. Conclusions

I have shown how the fluid mosaic model was an answer to the rise of molecular biology. The previous membrane models were unable to explain the capacity of cells to respond to their environment, and to communicate with other cells, in particular during development. One example will illustrate the limits of the previous models. In 1959, Frank MacFarlane Burnet had proposed the selective model of antibody production: cells of the immune system bear at their surface antibodies whose interaction with the corresponding circulating antigens activates the proliferation of these cells, and the secretion of antibodies. The Danielli-Robertson model was unable to provide any mechanistic description of these phenomena. This explanatory vacuum justified the proliferation of research as well as of reviews on membrane structure and function in specialized publications at the end of the 1960s (such as in *Annual Review of Biochemistry*). The structural and functional description of cell membranes progressively took the place occupied before by nucleic acids.

But the membrane model that emerged differed from that expected by molecular biologists. The organization of the

membranes was not (directly) inscribed in the genome, or in the proteins that the genome encodes. Membranes do not result from simple self-assembly of their constituent proteins.

The fluid mosaic model is emblematic of the new vision that emerged at the beginning of the 1970s, in which the precise description of molecular mechanisms is dovetailed with an integrated vision of cells and organisms. Membranes do exist as such, and not as aggregates of proteins. The rapid expansion of cell biology in the same years, supported by the development of new technologies such as indirect immunofluorescence, corresponded to the abandonment of a hard form of reductionism heralded by some of the leaders of molecular biology in which the only acceptable explanations are bottom-up (Morange 1997).

The new model did not drive out the previous model: it is a synthesis, a bringing together of various observations and different models. Nevertheless, this synthesis was something radically new, a revolution in this field of research. Historians and philosophers of science have paid more attention in the development of scientific knowledge to processes of replacement – replacement of a paradigm, a theory or a model by a new one – than to these processes of synthesis. They probably often occur in science, but they have not been looked for with enough scrutiny. The rise of the 'Modern Synthesis' in evolutionary biology and the emergence of the 'oncogene paradigm' (Morange 1993) are good examples of major transformations of scientific knowledge by synthesis.

Synthesis associates old and new models, old and new observations. It allows for the 'longue durée' (Holmes 2003) of some scientific concepts, instead of the frantic replacement of 'old' explanations and concepts by new ones.

These periods of synthesis are also fruitful times of transdisciplinarity. I have only been able to provide an impoverished description of the diversity of techniques, methods and disciplines that contributed to the emergence of the fluid mosaic model. Electron microscopists, specialists in optical rotatory dispersion and protein biochemists had little in common in their knowledge, and even less in their practice. They experienced difficulties appreciating the results obtained by their colleagues, and in estimating their strengths as well as their weaknesses. This did not, however, prevent the rapid elaboration of the fluid mosaic model of membranes, which remains valid after forty years of existence.

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