

# Gram-Negative Bacteria: “Inner” vs. “Cytoplasmic” or “Plasma Membrane”: A Question of Clarity rather than Vocabulary

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## Abstract

In this short commentary, we show that the utilisation of the term “inner membrane” to characterize the cytoplasmic or plasma membrane of Gram-negative bacteria can be a source of confusion and we propose that it should be completely abandoned.

## Letter

As explained in biochemistry or cell biology textbooks, all living cells are surrounded by a cytoplasmic (or plasma) membrane, containing phospholipids, proteins and other components that can be characteristic of the type of cell or organism. Sometimes, a more rigid, net-like cell wall constitutes an additional protective layer and lies outside the cytoplasmic membrane, for examples, peptidoglycan in bacteria and cellulose in plants.

In the bacterial world, the cell envelope of Gram-negative bacteria consists of two membranes that sandwich a layer of peptidoglycan (diderm bacteria), in contrast to that of Gram-positive bacteria where a thicker layer of peptidoglycan surrounds a single membrane (monoderm bacteria). Many authors refer to the two membranes of the Gram-negative bacteria as the “outer” and “inner” membranes, respectively. However, we argue that the utilisation of the term “inner” membrane does not describe the properties of this membrane adequately and represents a source of confusion. A striking example of this confusion is found in a paper by James Lake on the evolutionary origin of double-membrane (diderm) bacteria that was published in Nature [1]. In this paper, the author proposes that “Gram-negative prokaryotes were formed as a result of a symbiosis between an ancient actinobacterium and an ancient clostridium”. This hypothesis has been analysed and criticised from both theoretical and methodological points of view [2,3]. Alternative models which do not involve endosymbiosis have also been proposed [2,4].

Our purpose is not to contribute additional arguments to the debate but to try to clarify a major confusion in Lake’s paper arising from the inadequate utilisation of the terms “inner” and “outer” membranes as mentioned above. Indeed, one can read in the third paragraph of the section entitled “The double-membrane prokaryotes” that, in the single-membrane prokaryotes (i.e. mainly in Gram-positive bacteria), “there is no inner membrane, and the peptidoglycan layer, located outside the cell, surrounds the outer membrane” [1]. The author subsequently explains that some functional systems, located in the “inner” membrane of Gram-negative bacteria, are found in the “outer” membrane of their Gram-positive counterparts (the quotes refer to the author’s nomenclature).

Careful observation shows that most Gram-positive bacteria have only one membrane (see however the remark about Corynebacterineae below) which is thus neither an “inner” nor an “outer” membrane. It is a cytoplasmic (or plasma) membrane just as the “inner” membrane of Gram-negatives. In both cases, this cytoplasmic membrane contains phospholipids and many proteins, some of them included in large functional systems such as the flagellar motors, the respiratory electron

transporting chains, components of the final steps of peptidoglycan biosynthesis and, in the case of phototrophic bacteria, the photosynthetic apparatus (with the possible exception of some cyanobacteria for which the continuity between the thylakoids and the cytoplasmic membrane remains a subject of discussion [5]). Many of these proteins contain membrane-embedded alpha helices. Although some phospholipids are present in the inner leaflet, the outer membrane of Gram-negatives has a completely different chemical composition [6]. It is highly asymmetric and contains some specific compounds such as Braun’s lipoprotein and glycolipids (among which, in the outer leaflet, the lipopolysaccharides) that are never found in the cytoplasmic membranes. Moreover, most outer membrane proteins exhibit beta barrel structures [6] that are never observed in other membrane proteins with the exception of those in the outer membranes of Corynebacterineae, mitochondria and chloroplasts [7-9].

Mycobacteria and other Corynebacterineae are Gram-positives which exhibit an outer membrane containing mycolic acid and other complex lipids [10], but see also ref 2 for additional models of outer cell envelopes in prokaryotes). In contrast to those of Gram-negatives, their outer membranes appear to be rather symmetrical [6]. Interestingly, some proteins embedded in this bilayer also exhibit a beta-barrel structure [11], probably the result of convergent evolution.

In consequence, it is very possible, as suggested by Gupta [2] that different pathways leading to an additional protective layer were discovered (or even re-discovered) independently by various types of bacteria. This author suggests that the very different outer membranes evolved as a response to antibiotic production by other microorganisms such as Actinomycetes. Indeed, the outer membrane not only represents a supplementary permeability barrier, but it also keeps defensive enzymes from diffusing away, giving these bacteria a clear advantage over those with only one membrane. The example of  $\beta$ -lactamases, which confer resistance to penicillin and other  $\beta$ -lactam antibiotics, is striking. Most Gram-positives secrete the enzyme in the outer medium

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where it constitutes a “collective” resistance weapon: even the non-producing strains can take advantage of the production of the enzyme by their neighbours. In anthropomorphic terms, Gram-negatives are more “clever” and “selfish”: the enzyme remains in the periplasm, between both membranes, where its concentration can reach very high values [12] but it constitutes an individual weapon which only protects the producing cells. In this respect again, the Gram-positive mycobacteria are rather similar to their Gram-negative counterparts: the mycolic-acid containing outer membrane presents a diffusion barrier to the secretion of the  $\beta$ -lactamase into the outer medium and most of the enzyme remains in the mycobacterial equivalent of the periplasmic space of Gram-negative bacteria.

In conclusion it is clear, as generally accepted, that the “inner” membrane of Gram-negatives corresponds to the cytoplasmic membrane of Gram-positives from both structural and functional points of view and we suggest that, for the sake of clarity, the term “inner membrane” be completely abandoned for all kinds of bacteria for which we propose the general utilisation of the unambiguous terms “cytoplasmic” or “plasma” membrane. It is interesting to note that in most biochemistry and microbiology textbooks (but not in the cell biology ones), the terms plasma or cytoplasmic membrane are consistently preferred to “inner” membrane when the envelope of Gram-negative bacteria is described. The term “inner membrane” is only justified in the cases of mitochondria and chloroplasts, for which “cytoplasmic membrane” is clearly not adequate.

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