Trends in Microbiology – Spotlight

Engineering *E.coli* to have a hybrid archaeal/bacterial membrane

Laura Villanueva[1*]

[1]NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, P.O. Box 59, 1797AB Den Burg, Texel, The Netherlands. Correspondence: laura.villanueva@nioz.nl

Bacteria and Archaea have membrane lipids with an opposite stereochemistry. The most plausible explanation for this differentiation implies an unstable heterochiral membrane stage. A recent study engineers an *E.coli* with a significant abundance of archaeal lipids showing higher robustness disproving heterochirality as the driving force for this differentiation.

Lipid membranes are essential building blocks defining the cell as well as having a key function in energy maintenance and other physiological processes. The lipid membrane is also one of the most characteristic traits distinguishing the three domains of life. Membrane lipids of Bacteria and Eukarya are composed of fatty acids linked to glycerol-3-phosphate (G3P) via ester bonds, while those of Archaea have isoprene-based alkyl chains linked by ether linkages to glycerol-1-phosphate (G1P), resulting in the opposite stereochemistry of the glycerol phosphate backbone [1]. The differentiation of these two types of lipid membranes is known as the 'lipid divide' [2]. This process is believed to have occurred during the evolution of the Last Universal Common Ancestor (LUCA) before the divergence of Bacteria and Archaea. What lead to the 'lipid divide' is still now a topic of discussion. Previous studies have proposed a non-cellular LUCA lacking a lipid membrane [e.g. 3] that later acquired the corresponding membrane lipids. Alternatively, other studies have suggested that LUCA had a stable lipid membrane including fatty acids and isoprenes with G3P and G1P, respectively [4]. There is also no explanation for the opposite stereochemistry (chirality) of the glycerol backbone in Archaea and Bacteria as the enzymes involved in their synthesis, glycerol-1-phosphate-dehydrogenase (G1PDH) in Archaea and G3PDH in Bacteria, are not phylogenetically related suggesting they arose independently. However, the most accepted hypothesis is that LUCA had a 'hybrid' heterochiral lipid membrane with G1P and G3P together with fatty acids and isoprenoids [5]. This hybrid heterochiral membrane was initially seen as unstable being this the evolutionary pressure leading to the 'lipid divide' to evolve towards stable homochiral membranes. The instability of a heterochiral membrane was later challenged by *in vitro* experiments in which liposomes composed by archaeal and bacterial lipids were more stable that their homochiral counterparts [6]. Later, other studies reproduced an *in vivo* heterochiral membrane by engineering the bacterium *E. coli*. However the production of archaeal lipids in those membranes were too low to determine the membrane stability and physiological effects on the host bacterium [7].

The recent paper by *Caforio et al*. [9] challenges the expected instability of a heterochiral membrane by reporting a high level of archaeal membrane lipids in a genetically modified strain of *E. coli*. The authors genetically engineered *E.coli* by upregulating the endogenous isoprenoid

synthetic pathway MEP-DOXP, as well as introducing and expressing several key genes of the archaeal lipid biosynthetic pathway. Among those, the geranylgeranyl disphosphate (GGPP) synthase catalyzing the synthesis of GGPP from the isopentenyl diphosphate and dimethylallyl diphosphate (building blocks obtained from the isoprenoid pathway); G1PDH to synthesize G1P; archaeal homologs of the geranylgeranylglyceryl (GGGP) and the digeranylgeranylglyceryl phosphate (DGGGP) synthases catalyzing the two ether bonds between the isoprenoid side chains, and a CDP-archaeol synthase replacing the phosphate group of the unsaturated DGGGP by CDP generating unsaturated CDP-DGGGP.

Caforio *et al*. [8] reports an increase of up to 30% of archaeal membrane lipids (*i.e.* archaetidylglycerol, AG) at the expense of the bacterial membrane lipid phosphatidylglycerol. Besides, the hybrid heterochiral membrane remained stable after serial transfers indicating that the significant presence of AG was not toxic for the bacterial cell. On the other hand, the cells expressing the archaeal lipid biosynthetic pathway had a different cell morphology being slightly more elongated than normal. Strong induction of the archaeal lipid biosynthetic pathway lead to major cell morphology changes with formation of budding appendages that were eventually released out of the cell. These isolated bulges were confirmed to be formed by both archaeal and bacterial membranes lipids discarding the possibility of segregation of archaeal membrane lipids. Most importantly, the heterochiral membrane cells were seen to have a higher tolerance to heat, freezing conditions and against organic solvents which demonstrates that those cells have a higher fitness that the control ones at least in certain conditions.

Another remarkable finding of this study is that in control experiments with absence of G1PDH, but in the presence of the rest of archaeal lipid biosynthetic genes, the archaeal AG lipids still had the archaeal lipid G1P stereochemistry. This suggests that the bacterium *E. coli* harbors a yet-unknown and unprecedented mechanism to synthesize G1P. This fundamentally challenges the segregation of the glycerol backbone stereochemistry in membrane lipids between Bacteria and Archaea which is no longer as defined as we used to think. In fact, previous studies also reported the absence of G1PDH in the genome of the archaeon *Archaeoglobus profundus* as an exception within the Archaea [9]. Unfortunately, the stereochemistry of its lipids has never been analyzed but in view of the results of Caforio *et al.* [8] it is possible that also Archaea have an alternative pathway to synthesize G1P for their membrane lipids. Recently, it has been shown that two uncultured archaeal groups, *i.e.* the marine euryarchaeota group and Lokiarchaeota, newly discovered descendants of the archaeal ancestor leading to eukaryotes, contain the archaeal lipid synthesis genes as well as those needed for bacterial-like fatty acid and ester-bond formation [10]. However, they also lack the G1PDH to synthesize the G1P backbone but rather produce G3P, which suggests they have the potential to synthesize hybrid heterochiral membranes. These discoveries, together with the study by Caforio *et al.* [8], suggest that heterochiral lipid membranes might not be an exception neither a unstable short transitional step in cell membrane evolution, but rather a more common mechanism that previously thought.

This study delivers an engineered *E. coli* model synthesizing a hybrid heterochiral lipid membrane which can be now further tested for biotechnological processes. Future studies should devote efforts on further addressing the physiological advantages of a hybrid heterochiral membranes in light of the implications in the evolution of membrane lipid acquisition in early life.

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