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Spotlight

Engineering *E. coli* to Have a Hybrid Archaeal/Bacterial Membrane

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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 engineered *Escherichia 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 membrane 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 led to the 'lipid divide' is still a topic of discussion. Previous studies have proposed a noncellular 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 that 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 and acting as an 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 of archaeal and bacterial lipids were more stable than their homochiral counterparts [6]. Later, other studies reproduced an *in vivo* heterochiral membrane by engineering the bacterium *Escherichia coli*. However the production of archaeal lipids in those membranes was too low to determine the membrane stability and physiological effects on the host bacterium [7].

The recent paper by Caforio *et al*. [8] 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. These included the geranylgeranyl disphosphate (GGPP) synthase catalyzing the synthesis of GGPP from 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. In addition, the hybrid heterochiral membrane remained stable after serial transfers, indicating that

the significant presence of AG was not toxic for the bacterial cell. However, the cells expressing the archaeal lipid biosynthetic pathway had a different cell morphology and were slightly more elongated than normal. Strong induction of the archaeal lipid biosynthetic pathway led 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 membrane 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 organic solvents, which demonstrates that those cells have a higher fitness than the control cells, at least in certain conditions.

Another remarkable finding of this study is that – in control experiments with the 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 for synthesizing 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 Archaea also have an alternative pathway for synthesizing G1P for their membrane lipids. Recently, it has been shown that two uncultured archaeal groups, that is, 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 genes needed for bacterium-like fatty acid and ester-bond formation [10]. They also lack G1PDH for synthesizing the G1P backbone, producing G3P instead, which suggests that 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 nor an 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 now be further tested for biotechnological processes. Future studies should devote efforts to further address the physiological advantages of hybrid heterochiral membranes in light of the implications of the evolution of membrane lipid acquisition in early life.

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Spotlight

An Update on the *Acinetobacter baumannii* Regulatory Circuitry

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***Acinetobacter baumannii* adapts to different environmental conditions by expressing complex regulatory circuitry. Recent studies revealed that this circuitry includes regulatory factors that control the emergence of distinct bacterial subpopulations, which are critical for the capacity of this pathogen to persist in medical settings and cause infections in compromised hosts.**

The opportunistic pathogen *Acinetobacter baumannii* persists and thrives within the host and its surrounding environment inside and outside of medical settings. Thus, as a facultative pathogen, *A. baumannii* senses and responds to a myriad of intra- and extracellular signals that allow it to adapt to distinct environmental and host lifestyles through the action of different transcriptional regulatory systems, some of which are represented in Figure 1. Unexpectedly, light, one of the most ubiquitous environmental signals, is one of the cues to which *A. baumannii* responds to via the