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No prokaryotic GPI anchoring

To the editor:

In a recent article[1] in *Nature Biotechnology*, Jung et al. describe the construction of a chimerical protein made of *Zymomonas mobilis* levansucrase and *Pseudomonas syringae* ice nucleation protein (Inp). The fusion protein is expressed in *Escherichia coli* and displays cell surface localization.

I will not discuss the general value of this work, as my knowledge of bacterial enzymes is limited. However, I am extremely surprised to see the first line of the abstract stating that the Inp is a glycosylphosphatidylinositol (GPI)-anchored protein in some Gram-positive bacteria. My concern is that no GPI anchoring has been described in prokaryotes so far, and although we are all open to new discoveries, this fact is broadly accepted by the GPI community. The authors cite a paper[2] where the Inp is described as a lipoglycoprotein. Kozloff et al. make the hypothesis for the presence of a lipidic modification on some N-terminal asparagine-linked mannose residues, which is an interesting membrane anchoring motif, but which is very different from the GPI structure. Indeed, the GPI moiety is composed of the basic structure (phosphoethanolamine-(mannose)$_3$-glucosamine-PI)[3], with possible ethanolamine or glycan branching. The GPI moiety is synthesized on the endoplasmic reticulum, and is transferred en bloc on the C-terminal residue of a protein with concommitant elimination of a signal peptide. Furthermore, Jung et al. do not proceed to a real characterization of the GPI, a work that is not always easy but is necessary before announcing the presence of the GPI biosynthesis machinery in a prokaryote.

In conclusion, I see no experimental data that allow Jung et al. to take for granted the presence of the GPI motif in bacteria. Although the work for the identification of novel membrane anchoring motifs is essential, one should be aware of the experimental requirements necessary for an authentic discovery of GPI anchoring in bacteria. Again, the purpose of their work is not in question, but their preliminary postulate on GPI anchoring is.

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Jung et al. reply:

Kozloff et al. identified phosphatidylinositol, which is a relatively rare component of the prokaryotic cell wall, as a major component of the ice-nucleating site on the outer surface of *P. syringae*[1]. They also suggested that the Inp is structurally very similar to the GPI anchor of eukaryotic cells[2-4]. They, in fact, showed that all the available enzymes used for probing the GPI anchor proteins in eukaryotes affected the ice-nucleating activity. For example, PI-specific phospholipase CII treatment can severely decrease the class A ice-nucleating activity of wild-type *Pseudomonas*, *Erwinia*, and recombinant *E. coli* cells, suggesting the presence of PI moiety in Inp. Mannose residues were predicted from the results that α- and β-mannosidase also affect adversely each type of ice-nucleating activity. Nitrous acid, which attacks the amide linkage between inositol and glucosamine, abolished completely the class A and B ice-nucleating activity, indicating the presence of glucosamine residue. Through the tentative identification of PI, mannose, and possibly glucosamine residues as components of ice nucleation structures, they suggested that Inp has functionally similar structure to the eukaryotic GPI anchor, although not necessarily identical in its chemical structure. The GPI anchor protein should be released from the surface by treating whole cells with the GPI- or PI-specific PLC(II), although some resistant forms are found[5]. This is the simplest and the most direct method. The Inp, however, was not released into the medium by PI-specific PLC(II) treatment, probably because the INP exists as an aggregate on the surface. We can now provide the complementing evidence for Kozloff et al.'s proposal of GPI-like Inp structure by making deletions of the N-terminal, repeating- or C-terminal domains of Inp. As these mutant Inps are expected to become more accessible to enzymes, we will determine whether Inp is released with PI-PLC(II). The N-glycosylated or O-glycosylated surface proteins have recently been found in archaea and bacteria[6], so it is not totally unexpected, at least for us, to have found phosphatidylinositol-anchored glycoproteins or GPI-anchored proteins in bacteria.

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