

Classic Spotlight: Gram-Negative Bacteria Have Two Membranes

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The Danish microbiologist Hans Christian Gram developed a staining technique (1884) that classifies most bacteria into two large groups that are referred to eponymously, as Gram-positive and Gram-negative bacteria (1). His stain is still used in medical microbiology labs today, and because of its explanatory power, this classification is the most commonly used descriptor of a particular bacterium in scientific reports. It has been clear since the beginning that the Gram stain must detect some fundamental difference in the cell envelopes of these two types of bacteria, but elucidating cell envelope structure, particularly in the case of Gram-negative bacteria, required 80 years and the development of thin-section electron microscopy.

In a classic *Journal of Bacteriology* paper, Bladen and Mergenhagen (2) showed clearly for the first time that unlike the cell envelopes of Gram-positive bacteria, which contain a thick peptidoglycan cell wall that surrounds a single membrane, the cell envelopes of Gram-negative bacteria are composed of three structural entities: an inner or cytoplasmic membrane, a thin, rigid cell wall, and an outer membrane. They demonstrated by using lysozyme that the structure between the two membranes is the peptidoglycan and by using selective extraction that the characteristic glycolipid found in Gram-negative bacteria, lipopolysaccharide, is present in the outer membrane. These authors coined the term “outer membrane.”

This work helped explain that the thick “cell wall” previously observed in electron micrographs of Gram-negative bacteria was

really tightly associated outer membrane and peptidoglycan. It also revealed an aqueous cellular compartment between the two membranes that is now called the periplasm, and this helped solve another paradox. Both Gram-positive and Gram-negative bacteria express degradative enzymes such as DNase, RNase, and alkaline phosphatase. In Gram-positive bacteria, these enzymes are secreted or attached to the cell surface. In Gram-negative bacteria, these enzymes are cell associated and soluble. They do no harm because they are confined to the periplasm (3).

REFERENCES

1. Gram HC. 1884. Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten. *Fortschr Med* 2:185–189.
2. Bladen HA, Mergenhagen SE. 1964. Ultrastructure of *Veillonella* and morphological correlation of an outer membrane with particles associated with endotoxic activity. *J Bacteriol* 88:1482–1492.
3. Wetzel BK, Spicer SS, Dvorak HF, Heppel LA. 1970. Cytochemical localization of certain phosphatases in *Escherichia coli*. *J Bacteriol* 104:529–542.

Citation Silhavy TJ. 2016. Classic spotlight: Gram-negative bacteria have two membranes. *J Bacteriol* 198:201. doi:10.1128/JB.00599-15.

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