

This Week's Citation Classic

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Muhlethaler K, Moor H & Szarkowski J W. The ultrastructure of the chloroplast lamellae. *Planta* (Berlin) 67:305-23, 1965.

The ultrastructure of chloroplast thylakoids was studied with the newly developed freeze-fracturing technique. In contrast to the classical methods used in electron microscopy large membrane areas could be studied in surface view. Specific patterns of protein particles were observed in this membrane system. [The *SCI*[®] indicates that this paper was cited 137 times in the period 1961-1977.]

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"Frequent citation of a paper may indicate either that a substantial contribution to understanding a biological process or structure was achieved, or that a new approach with a better technique was taken. In our paper both aspects were involved. To me, as a biologist, the idea put forward in 1960 by Robertson, that all living organisms in their various cells should have the same tripartite membrane, seemed too simple. It struck me as more reasonable to assume that different structural (and functional) arrangements of lipids and proteins had arisen during evolution.

"As an electron microscopist of the pioneering days of the art, I had become dissatisfied with a situation, in which the use of the EM to reveal membrane structure was based entirely on studies of fixed and sectioned material. The same year that Robertson postulated his "unit membrane," work was going on in our laboratory to develop

an alternative method for specimen preparation in the frozen state. We soon recognized that this method, which we called freezeetching, was well suited for membrane studies, because fracturing occurred preferentially along cellular membrane systems, permitting extended surface views of the membranes.

"It became apparent that membranes are composed of an amorphous sheet, covered with particles of various sizes arranged in specific patterns and distributions. Some of these particles could be washed off in dilute buffer solutions, whereas the remaining ones seemed partly embedded in the membrane matrix. Based on these findings we postulated, in our 1965 paper, a model of the thylakoid membrane, consisting of a lipid bilayer in which globular protein complexes were embedded. For their geometry, these particles appeared able to bridge the lipid layer contacting proteins on the opposite side of the membrane. The possible formation of protein bridges seemed potentially important for a better understanding of transport processes.

"In our 1965 study of thylakoids we intended to demonstrate the potential of the various electron microscopic techniques for membrane studies in general. With the combined use of freeze-fracturing, standard thin sectioning, and negative contrast, we came to a more comprehensive view of the architecture of cellular membrane systems than previously obtained. Freeze fracturing in combination with other techniques is now standard. Probably because the article appeared in a botanical journal, the aspects important for understanding membrane structure in general were overlooked at first. It is gratifying to see that our work has now been generally accepted."