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This Week's Citation Classic

Ryter A & Kellenberger E. Etude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoides des bactéries en croissance active. (Electron microscope study of plasmas containing DNA. I. Bacterial nucleoids in active growth.)
Z. Naturforsch. Sect. B 13:597-605, 1958.
[Laboratoire de Biophysique, Université de Genève, Switzerland]

The influence of fixation conditions on the bacterial nucleoid fine structure was studied by varying different parameters. It could be concluded that the finest fibrillar structure of the nucleoplasm was obtained when fixation was performed with one percent osmium tetroxide dissolved in Michaelis buffer pH 6.0 containing Ca^{++} and amino acids followed by post-fixation with uranyl acetate. [The *SCI*® indicates that this paper has been cited in over 890 publications since 1961.]

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"This work was performed between 1953 and 1958 in the biophysics laboratory of the University of Geneva (Switzerland), which was directed by E. Kellenberger. At that time, electron microscopy and especially ultrathin sectioning were just beginning to be used. The osmium fixation conditions generally used for animal cells gave unsatisfactory results in bacteria, especially for nuclear material. The latter generally presented the appearance of rather coarse filaments. This pushed us to study carefully the influence of fixation conditions on the nucleotide fine structure and different DNA plasmas.

"This study was long and fastidious because several parameters intervened and interfered with each other. It finally appeared that phosphate buffer, currently used for eukaryotic cells, had to be absolutely avoided because calcium ions were indispensable for a good preservation. The slightly acidic pH, the presence of amino acids or small peptides, and at last the post-fixation with uranyl acetate all appeared to be necessary for a reproducible and good preservation of the nucleoid.

"These peculiar conditions are not required by eukaryotic nuclei because the histones present on DNA are rapidly cross-linked by the fixative, and maintain the three-dimensional structure of the chromosome. The fibrillar structure of the bacterial chromosome suggests that DNA association with protein partners, if it exists, is certainly much more discrete or labile than that of nucleohistones and is not sufficient to prevent DNA from collapsing during alcohol or acetone dehydration.

"One can add that this technical study was complicated by the fact that methacrylate, which was used at that time as embedding medium, frequently produced swelling and even 'explosion' of bacteria. To overcome this artifact we searched for a better embedding medium (a polyester[1]) which was improved at the same time as fixation. It is interesting to point out that this swelling artifact in methacrylate, which was especially obvious in bacteria, also led A.M. Glauert to find another embedding medium (Araldite).[2]

"The association of good fixation conditions and an improved embedding medium allowed Kellenberger and his collaborators to make major advances in the knowledge of the bacterial nucleoid[3] and to obtain, for the first time, information on intracellular bacteriophage multiplication.[4] The frequent citation of this paper is due to the fact that these fixation conditions were used by almost all electron microscopists working on prokaryotic cells. It is also sometimes only cited for post-fixation with uranyl acetate, which is often applied to eukaryotic cells because it improves membrane contrast and clearly shows its triple-layered structure."

1. **Kellenberger E, Schwab W & Ryter A.** L'utilisation d'un copolymère du groupe des polyesters comme matériel d'inclusion en ultramicrotomie? *Experientia* 12:421-2, 1956.
2. **Glauert A M & Glauert R H.** Araldite as an embedding medium for electron microscopy. *J. Biophys. Biochem. Cytol.* 4:191-4, 1958.
3. **Kellenberger E, Ryter A & Séchaud J.** Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. *J. Biophys. Biochem. Cytol.* 4:671-8, 1958.
[Citation Classic. *Current Contents/Life Sciences* 23(7):12, 18 February 1980.]
4. **Kellenberger E, Séchaud J & Ryter A.** Electron microscopical studies of phage multiplication. IV. The establishment of the DNA pool of vegetative phage and the maturation of phage particles. *Virology* 8:478-98, 1959.