Preparation conditions for optimal ultrastructural preservation and for immuno-labeling in TEM analysis of Archaea

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Keywords: high-pressure freezing, freeze-substitution, immuno-labeling, fracture-labeling

In the course of on-going investigations on the ultrastructure of Archaea and the subcellular distribution / localization of proteins, we have used a variety of techniques for optimizing the results. The cells investigated in these studies are (hyper-)thermophilic Archaea of a variety of genera and species. The results presented will focus on experiments performed with cells of the genera *Pyrodictium*, *Ignicoccus* and *Nanoarchaeum*, although other archaeal cells were also investigated [1].

In all cases, we cultivated the cells in cellulose capillary tubes [2,3], resulting in unexpected high cell densities. Thus, we were able to avoid centrifugation steps, as commonly used for concentrating cells, but which are deleterious for preserving cell-cell interactions [1] commonly found in these Archaea. Next, cells are immobilized by high-pressure freezing, avoiding any chemical fixation at room temperature. The following step is freeze-substitution-fixation: cellular and extracellular water is substituted by organic solvents (usually acetone or ethanol) at low temperatures. Concomitantly, cells are subjected to low temperature chemical fixation, usually with aldehydes, and contrasted by low amounts of heavy metal compounds (Uranyl acetate, occasionally also OsO_4). For this step, more than 10 different "cocktails" were tested, all yielding good results, with minor but important variations in the degree of structural preservation of intracellular filaments (in *Ignicoccus* cells; Fig. 1) [4]. Finally, samples are embedded in a resin at room temperature. We use Epon resin for both, ultrastructural analysis and immuno-labeling, with good success rates. Immuno-labeling is done on sections, and involves all commonly used steps, including secondary antibodies with the smallest labels available (6nm, ultrasmall or nano gold; Fig. 2), and silver-enhancement with protocols available in the literature [5].

In some cases, we also employed fracture-labeling for localizing membrane epitopes on freeze-fractured cell membranes [6], a method which offers access to larger amounts of antigenic sites, when compared with ultrathin sections.

Finally, we also attempted to localize a protein in long, extracellular appendages, commonly referred to as "flagella"; these fibres turned out to be structures which functionally serve as adhesion organelles. The successful labeling was, unexpectedly, only possible after treating the fibres under harsh conditions.

Summarizing, the results show that the first step – the cultivation – turned out to be crucial for the interpretation of the final results. Cryopreparation was absolutely mandatory for preserving the delicate, subcellular fine structure. Epon resin yields best results in structural preservation of the cells, as shown in other studies; in addition, it does permit successful post-embedding immuno-labeling on sections for a variety of proteins, cytosolic and membrane, at least for these cells.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-234, © Verlag der TU Graz 2009

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7. This research was supported by the Deutsche Forschungsgemeinschaft. The aid of Gertraud Rieger, Sabine Riehl and Cordula Neuner is gratefully acknowledged.

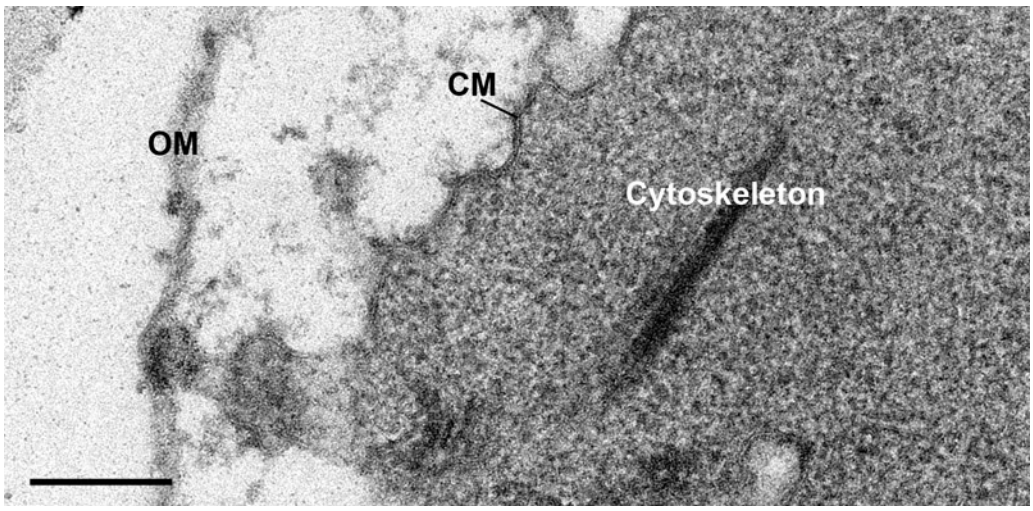

Figure 1. Transmission electron micrograph of an ultrathin section of *Ignicoccus hospitalis*, following cultivation in cellulose capillaries, high-pressure freezing, freeze-substitution and embedding in Epon. CM, cytoplasmic membrane; OM, outer membrane. Bar, 200 nm.

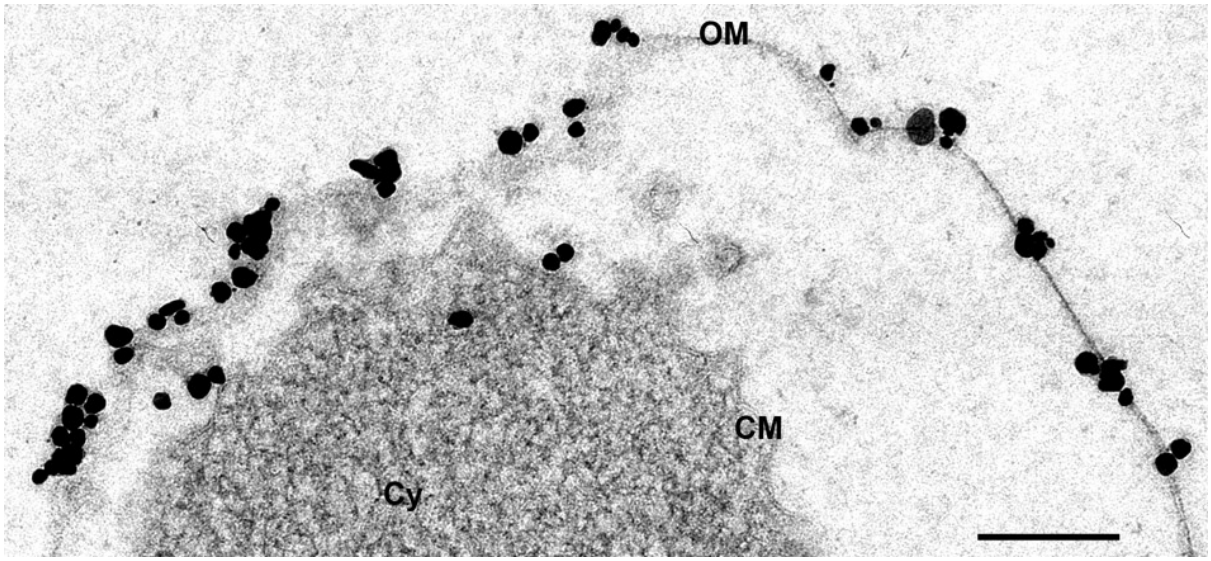

Figure 2. Transmission electron micrograph of an ultrathin section of *Ignicoccus hospitalis*, prepared as described for Fig.1. Immuno-labeling with a specific antibody raised against the major outer membrane protein Ihomp1 [6]. Secondary antibody with ultrasmall gold, followed by silver enhancement [5]. Bar, 200 nm.

DOI: 10.3217/978-3-85125-062-6-234,