Ultrastructural & Analytical Methods in Life Sciences

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Immunolabelling on sections in 2D and 3D, with speed and ecstasy

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Antibody incubation steps in commonly used protocols for immunolabeling on ultrathin sections usually last for one hour or two hours. Here we show that shorter incubation times (down to 5 min) are absolutely sufficient and moreover, help to better preserve structural details. For this, we labeled ultrathin sections of cells of the autotrophic, anaerobic, hyperthermophilic Crenarchaeon *Ignicoccus* [1], embedded in Epon after high-pressure freezing (HPF) and freeze-substitution fixation (FSF) (Figure 1). Beside our methodical interest in speeding up the labeling protocol, our biological interest was in the localization of enzymes of the unique CO_2-fixation pathway - the dicarboxylate/4-hydroxybutyrate cycle. This also includes an enzyme converting 4-hydroxybutyrate, which is also known as "liquid ecstasy" (GHB), into 4-hydroxybutyryl CoA [2].

Among Archaea, *Ignicoccus* cells exhibit an extraordinary ultrastructure. In addition to a cytoplasmic membrane, there is an outer cellular membrane (OCM), which encases an intermembrane compartment (IMC). The IMC contains huge amounts of vesicles or tubes [3]. Another curiosity is that the archaeal ATP synthase is located in the OCM [4]. From this structural and functional compartmentalization of the cells, the question arose about the subcellular distribution of enzymes involved in different steps of the CO_2 fixation pathway.

Using our accelerated immunolabeling protocol, we could detect the acetyl-CoA-synthetase in association with the OCM and the 4-hydroxybutyryl-CoA-synthetase (the key enzyme) in the cytoplasm. For investigating spatial distribution we generated 3D-models on the basis of immunolabeled serial sections (Figure 2). From our results, it becomes evident that the CO_2 fixation takes place in different cell compartments. Thus, we are currently about to target further proteins of this CO_2-fixation pathway, to track down its route and get a deeper understanding in the physiology of these highly unusual cells.

1. H. Huber et al., PNAS 105 (2008), p. 7851
2. U. Jahn et al., J. Bacteriol. 189 (2007), p. 4108
3. R. Rachel et al., Archaea 1 (2002), p. 9
4. U. Küper et al., PNAS 107 (2010), p. 3152
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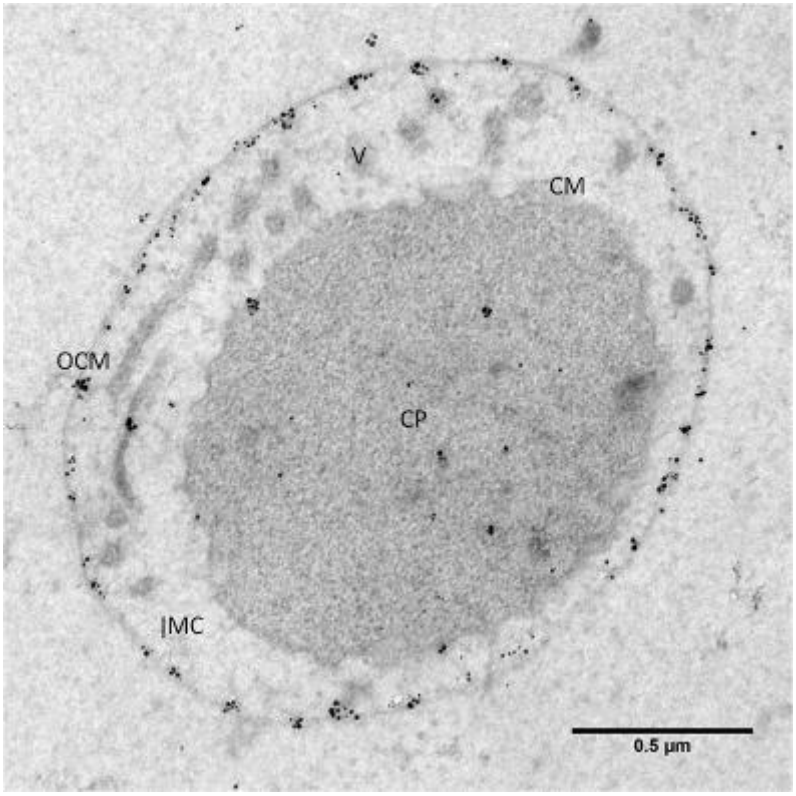

Figure 1. Ultrathin section of *Ignicoccus*. Cell was cryo-fixed, freeze-substituted, and embedded in Epon. Section was labelled with antibodies directed against the ATP synthetase; incubation time of antibodies 25 min; detection with goat anti-rabbit immunoglobulin “ultrasmall-gold”; bar, 0.5 µm.

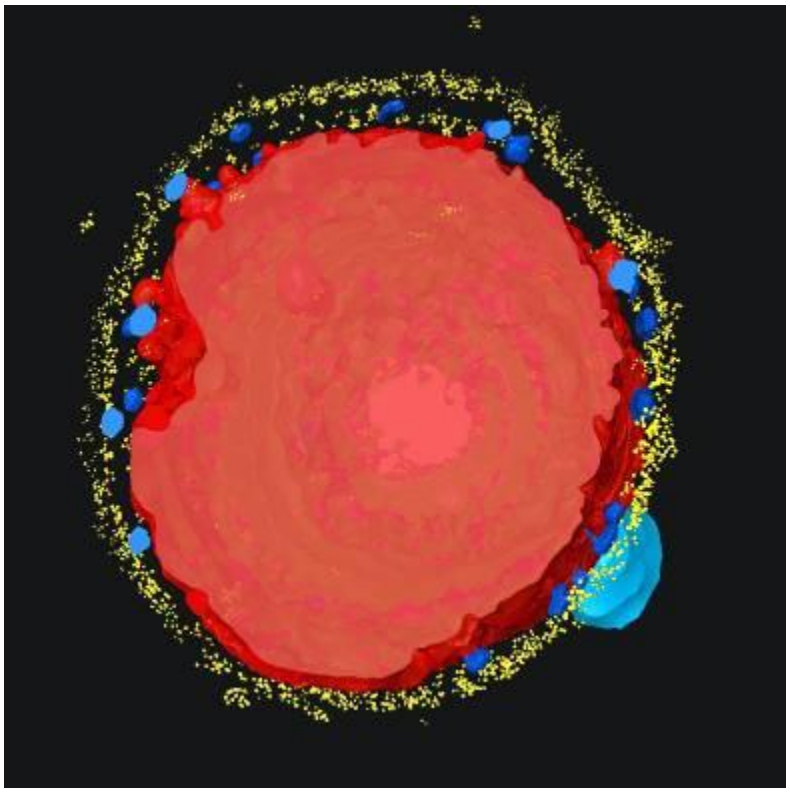

Figure 2. 3D-reconstruction and visualization of a data set of serial sections from an *Ignicoccus* cells, prepared as described, and labeled with antibodies directed the Acetyl-CoA synthetase. Alignment, segmentation, and visualization were done using AMIRA. Red: cytoplasm; blue: vesicles in the IMC; yellowish/white: gold; light blue: *Nanoarchaeum*.