Isolation of a “prokaryotic cell organelle” from the uniquely compartmentalized anammox bacteria

S Neumann¹, MSM Jetten¹ and L van Niftrik¹

1. Department of Microbiology, Institute for Water & Wetland Research, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

email: s.neumann@science.ru.nl
keywords: anaerobic ammonium oxidation; bacteria; cell compartmentalization; cell organelle isolation

Prokaryotic cells that belong to the domain Bacteria, are commonly subdivided into a Gram-positive and a Gram-negative type based on the organization of their cell wall. However, some bacteria defy this traditional classification. One of the most notable phyla in this respect is the Planctomycetes. They are thought to be devoid of several characteristics associated with Gram-positive or Gram-negative bacteria: a periplasmic space, a peptidoglycan-containing cell wall or an outer membrane [1]. In addition, the planctomycete cell is subdivided into two compartments. The outermost compartment, the paryphoplasm, is thought to be cytoplasmic and to be surrounded by the cytoplasmic membrane. This compartment is separated by an internal lipid bilayer membrane from the riboplasm, which contains the DNA, RNA and ribosomes of the cell [2].

Bacteria conserve the energy required for growth and maintenance of the cell by oxidation of organic or inorganic compounds. Although thermodynamically favorable, the oxidation of ammonium with nitrite as electron acceptor was not known to be facilitated by any organism, until a little more than a decade ago the anammox (anaerobic ammonium oxidizing) bacteria were discovered [3]. They are members of the Planctomycetes, very slow growers and have been found in many different habitats around the world. Their importance for the biogeochemical cycling of nitrogen on a global scale is now recognized and they are currently applied for the cost-efficient and environment-friendly removal of nitrogen compounds from wastewater [4, 5, 6]. Aside from their metabolism, anammox bacteria presented another novelty in the bacterial world. A great volume of the anammox cell is taken up by a central cell compartment, which has not been observed in other planctomycetes and has been named the anammoxosome [7]. The cell of anammox bacteria is therefore subdivided into three compartments: on the outside the paryphoplasm that surrounds the riboplasm, and the most central anammoxosome (Figure 1). This cell plan is present in all anammox bacteria known so far. Based on previous studies that used transmission electron microscopy (TEM) combined with immunogold-labeling to localize key metabolic proteins [2, 8] and peroxidase staining for localization of cytochrome c proteins [9], it is hypothesized that the anammoxosome is a “prokaryotic cell organelle” harboring the energy metabolism of the cell. Our understanding of the cell ultrastructure is, however, still incomplete. The function of the paryphoplasm is elusive, except for being the location of the cell division ring [10], and the central role of the anammoxosome in the catabolism of the cell has not yet been directly demonstrated. The latter is due to the fact that the anammoxosome has not been isolated from the rest of the cell in sufficient quantities and purity for conclusive experiments.

Our aim was to gain further insight into the function of the anammoxosome and the cell compartmentalization in anammox bacteria by isolating the anammoxosome from the anammox cell. Physical and chemical cell disruption techniques were combined and the resulting subcellular fractions were separated from each other by density gradient centrifugation. Immunofluorescence analysis was used to investigate the fractionated anammox cells for their shape, DNA content and the hybridization with an anammoxosome-specific antibody. After chemical fixation or cryo-fixation with subsequent freeze-substitution, ultra-thin sections were evaluated by TEM in order to evaluate the intactness of the different cellular membranes. Additionally, cryo-scanning electron microscopy (cryoSEM) was performed. The ability of the different subcellular fractions to perform the anammox reaction was tested and their proteome was analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
References


Figure 1. The ultracellular organization of the anammox bacterium “Candidatus Kuenenia stuttgartiensis”; ICM: intracytoplasmic membrane; AM: anammoxosome membrane; scale bar: 200 nm [11]