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Linking ultrastructure and function in the uniquely compartmentalized anammox bacteria

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Prokaryotic cells belonging 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*. These bacteria are thought to be devoid of several characteristics associated with Gram-positive or Gram-negative bacteria: a periplasmic space, a peptidoglycan-containing cell wall and an outer membrane [1]. In addition, the planctomycete cell is subdivided into two compartments: the outermost compartment – the paryphoplasm – is thought to be surrounded by the cytoplasmic membrane, but its function is so far unknown. An internal lipid bilayer membrane separates the paryphoplasm from the riboplasm, which contains the DNA, RNA and ribosomes of the cell [2].

The anammox (**anaerobic ammonium oxidizing**) bacteria belong to the phylum *Planctomycetes*. Prokaryotic organisms 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 the anammox bacteria were discovered [3]. Anammox bacteria 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 eco-friendly removal of nitrogen compounds from wastewater [4, 5, 6]. Aside from their metabolism, anammox bacteria presented another novelty in the bacterial world: the majority of the 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]. Anammox bacteria are therefore subdivided into three compartments: the cell is rimmed by the paryphoplasm that surrounds the riboplasm, and innermost lies the anammoxosome (Figure 1). This cell plan is present in all anammox bacteria discovered 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. In particular the function of the paryphoplasm remains elusive, except for being the location of the cell division ring [10], and the central role of the anammoxosome in the catabolism of the anammox 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. The isolation by itself provided strong evidence that anammoxosomes are truly independent “cell organelles”. They were then used in biochemical assays in order to show the conversion of anammox substrates by isolated anammoxosomes outside of their regular cellular context, since this would corroborate that they harbor the energy metabolism of the anammox cell. Furthermore, proteome analysis of isolated anammoxosomes substantiated their hypothesized role in energy conservation and might also lead to new insights about the anammoxosome. Finally, we located several enzymes of interest, i.e. proteins with possible structural functions as well as enzymes involved in the energy metabolism, inside the anammox cell by immunogold localization.

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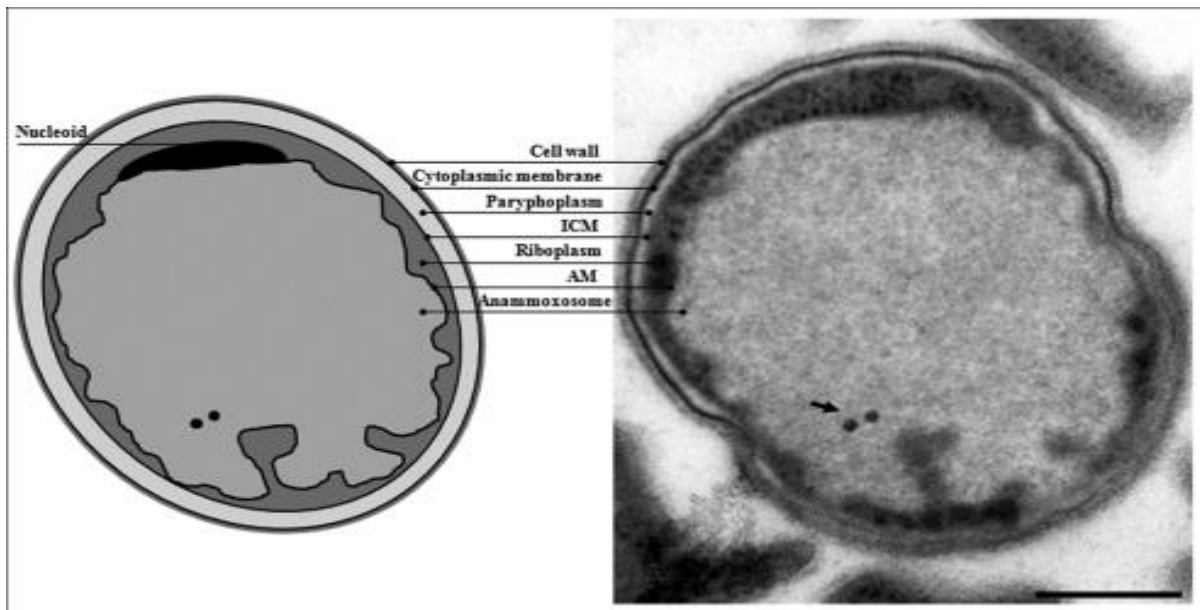


Figure 1. The ultrastructural organization of the anammox bacterium "*Candidatus Kuenenia stuttgartiensis*"; ICM: intracytoplasmic membrane; AM: anammoxosome membrane; scale bar: 200 nm [11].