LS-2-P-1657 Three-dimensional reconstruction of Angomonas deanei reveals association of the protozoa organelles and a symbiotic bacterium

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Co-evolution between primitive organisms associated by symbiotic relationship can offer valuable information about the origin of organelles and the evolution of the eukaryotic cell. Some monoxenic protozoa from the Trypanosomatid family, as Angomonas deanei, maintain a mutualistic relationship with a bacterium that divides in synchrony with other host cell structures. In this work we performed structural analysis using high-pressure freezing and freeze substitution associated to the electron tomography technique in order to study the symbiont ultrastructure and its association with protozoan organelles. Such analyses were combined with genomic data obtained from the bacterium and its host Trypanosomatid. Our results showed a close association of the symbiont with the host nucleus, reinforcing the idea that the protozoan controls the bacterium division and that during this process the nucleus serves as a topological reference to the symbiont segregation. During the protozoan cytokinesis, the bacterium maintains its position close to the nucleus ensuring its inheritance to each daughter cell. Interestingly, our genomic data showed that the symbiont presents a reduced content (±830 kb), indicating a massive gene loss as those from the dcw (division and cell wall) cluster. These sequences code proteins of the bacterial division ring, known as Z-ring and those involved in peptidoglycan layer synthesis and septum formation. Such structures are not detected in the symbiont envelope after using classical optical and electron microscopy techniques. In this work, the use of electron tomography membrane contrast revealed a detailed ultrastructure of the symbiont envelope that presents a reduced cell wall and lacks the Z ring. Another interesting aspect of this symbiotic relationship is the intense metabolic exchange between the associated partners, as the symbiont obtains phosphatidylcholine from the host Trypanosomatid. According to this idea the high-resolution microscopy showed that the endoplasmic reticulum presents an intimate relationship with the bacterium, with some contact points observed between these structures. Taken together, our results assume that an ultrastructural and metabolic association between the symbiont and host cell structures is essential to maintain the endosymbiosis in trypanosomatids.

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Fig. 1: (A-C) FIB-SEM. Symbiont (green); Protozoa nucleus (blue); Glicossomes (yellow); ER (red) (D-E) Electron Microscopy. Symbiont (S); ER (arrow); Nucleus (N); Glicossome (G). (F) Kinetoplast (K) in pink. (G-H) Cryo-techniques were used to verify membrane fusion events. Mithochondrion (M) in yellow; ER (red); Symbiont (green). Scale bar: 0.5 µm.