ULTRASTRUCTURAL STUDIES OF TWO MODEL MINIMAL CELLS BY ELECTRON CRYOTOMOGRAPHY

Thesis by

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Abstract

While most motile bacteria propel themselves with flagella, other mechanisms have been described including retraction of surface-attached pili, secretion of polysaccharides, or movement of motors along surface protein tracks. These have been referred to collectively as forms of "gliding" motility. Despite being simultaneously one of the smallest and simplest of all known cells, *Mycoplasma pneumoniae* builds a surprisingly large and complex cell extension known as the attachment organelle that enables it to glide. Here, three-dimensional images of the attachment organelle were produced with unprecedented clarity and authenticity using state-of-the-art electron cryotomography. The attachment organelle was seen to contain a multi-subunit, jointed, dynamic motor much larger than a flagellar basal body and comparable in complexity. A new model for its function is proposed wherein inchworm-like conformational changes of its electron-dense core are leveraged against a cytoplasmic anchor and transmitted to the surface through layered adhesion proteins.

The hallmark of eukaryotic cells is their segregation of key biological functions into discrete, membrane-bound organelles. Creating accurate models of their ultrastructural complexity has been difficult in part because of the limited resolution of light microscopy and the artifact-prone nature of conventional electron microscopy. Here we explored the potential of the emerging technology electron cryotomography to produce three-dimensional images of an entire eukaryotic cell in a near-native state. *Ostreococcus tauri* was chosen as the specimen because as a unicellular picoplankton with just one copy of each organelle, it is the smallest known eukaryote and was therefore likely to yield the highest resolution images. Whole cells were imaged at various stages of the cell cycle, yielding 3-D reconstructions of complete chloroplasts, mitochondria, endoplasmic reticula, Golgi

bodies, peroxisomes, microtubules, and putative ribosome distributions *in-situ*. Surprisingly, the nucleus was seen to open long before mitosis, and while one microtubule (or two in some predivisional cells) were consistently present, no mitotic spindle was ever observed, prompting speculation that a single microtubule might be sufficient to segregate multiple chromosomes.

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