

**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.

Table of Contents

ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
TABLE OF CONTENTS.....	vi
LIST OF TABLES AND FIGURES.....	viii
Chapter 1: Introduction.....	1
Minimal Cells.....	1
Electron Microscopy and Sample Preservation.....	2
Mycoplasmas and the Attachment Organelle.....	5
Eukaryote Ultrastructure.....	10
Personal Contributions.....	14
References.....	16
Chapter 2: Three-dimensional structure of <i>Mycoplasma pneumoniae</i> 's attachment organelle and a model for its role in gliding motility.....	II-1
Abstract.....	II-2
Introduction.....	II-3
Results.....	II-5
Discussion.....	II-9
Experimental Procedures.....	II-14
Acknowledgements.....	II-15
References.....	II-16
Figures	II-21
Chapter 3: Three-dimensional ultrastructure of <i>Ostreococcus tauri</i> : electron cryotomography of an entire eukaryote cell.....	III-1
Summary.....	III-2
Introduction.....	III-3
Results and Discussion.....	III-5

Material and Methods.....	III-26
Acknowledgements.....	III-29
References.....	III-30
Figures and Video.....	III-39
Chapter 4: Conclusion.....	IV-1
Reference.....	IV-2
Appendix A: Electron cryotomography sample preparation using the Vitrobot.....	A-1
Abstract.....	A-2
Introduction.....	A-3
Materials.....	A-6
Procedure.....	A-11
Anticipated Results.....	A-19
References.....	A-20
Figures and Tables.....	A-23

List of Tables and Figures

Figure II-1. Electron micrograph and tomographic reconstruction of a dividing <i>M. pneumoniae</i> cell.....	II-21
Figure II-2. Montage of attachment organelles and schematic.....	II-22
Figure II-3. Extracellular surface proteins.....	II-23
Figure II-4. Membrane proteins and terminal button.....	II-24
Figure II-5. Electron-dense core.....	II-25
Figure II-6. Bowl complex.....	II-26
Figure II-7. Multiple electron-dense cores.....	II-27
Figure II-8. Cytoskeleton filaments.....	II-28
Figure II-9. Evidence of conformational changes.....	II-29
Figure III-1. Cross-section and whole cell segmentation of <i>O. tauri</i>	III-39
Figure III-2. Light microscope images of free-floating <i>O. tauri</i>	III-41
Figure III-3. Whole cell segmentation of six cells imaged at the dark-to-light transition (mid G ₁).....	III-42
Figure III-4. Cells with dividing organelles.....	III-43
Figure III-5. Chloroplast.....	III-44
Figure III-6. Dynamics of the nuclear envelope.....	III-45
Figure III-7. Close-up view of a nuclear pore complex.....	III-47
Figure III-8. Mitochondrion.....	III-48
Figure III-9. Endoplasmic reticulum.....	III-49
Figure III-10. Golgi body.....	III-50
Figure III-11. Microtubule.....	III-52
Figure III-12. Ribosome-like complexes.....	III-53
Figure III-13. External protein complexes.....	III-54
Figure III-S1. Preservation of <i>O. tauri</i>	III-55
Figure III-S2. Cross-correlation curves.....	III-56

Figure A-1. . Example image showing well-preserved bacterial cells, a good distribution of gold fiducials, and thin ice.....	A-23
Table A-1 Vitrobot blotting parameters for different samples.....	A-24
Table A-2. Troubleshooting	A-25