MICROTUBULES: THE RHYTHM OF ASSEMBLY AND THE EVOLUTION OF FORM

by

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ABSTRACT

The subject of this thesis is an unusual protein polymer, essential to life in all eukaryotic (nucleus-bearing) cells, known as a *microtubule.* Microtubules are especially interesting because they exhibit *dynamic instability,* a process in which chemical energy (GTP-hydrolysis) is converted into macroscopic length fluctuations.

Tubulin, the constituent protein of microtubules, was purified from cow brain. Its assembly into microtubules was observed in real time under the light microscope using video-enhanced DIC microscopy. The chemical condition of the aqueous environment and the temperature were varied.

This thesis presents the phase diagram of microtubule self-assembly and length dynamics over a wide range of temperatures (10-40°C) and tubulin concentrations (6-60μM). Both heterogeneous and homogeneous nucleation are investigated and a qualitative transition in the length dynamics, from bounded to unbounded growth, is documented. In addition, the stabilizing effect of glycerol is explored. Of the many observations, the effect of temperature on the frequency of fluctuations between assembly and disassembly is particularly noteworthy since it is not explicable by current models for dynamic instability. A new model is called for in which destabilization of the microtubule is uncoupled from the growth rate at low temperatures and strongly coupled to it at high temperatures. The intermittent "zippering" of a tubulin sheet into a microtubule is suggested as a possible destabilizing mechanism.

The experimental control over microtubules given by the phase diagram permits the use of microtubules far from their native environment. In the second part of this thesis, microtubules are used to create a simple model system for studying biological morphogenesis and force generation.

Tubulin is encapsulated within phospholipid vesicles at low temperatures. Microtubules assemble when the temperature is raised and the vesicles are observed under the light microscope. The microtubules deform near-spherical membranes through a specific, reversible and unusual sequence of shapes. Their polymerization exerts a force of several picoNewtons on the membrane which, in turn, often results in the buckling of the microtubules themselves. The possibility of a thermal ratchet mechanism for force generation is discussed.

Advisor: Professor Albert Libchaber

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Dear Reader, Please do not take the fact that only one name claims authorship of this work to mean that it was done alone. Nothing could be further from the truth. It has fluorished in cooperation with many generous and caring people around me, for whose efforts and patience I am deeply grateful. If this thesis stimulates you to think about an unfamiliar subject or about a familiar one in a new way, you will have a glimpse of the challenges that have been my lucky fate these past four years, and the writing of this thesis will not have been in vain.

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