

The Role of Actin and Myosin in the Formation of Microtubule System during Differentiation of *Naegleria gruberi*.

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Microfilaments and microtubules are the two dynamic components of the eukaryotic cytoskeleton and participate in various cellular activities in concert. These observations suggest intimate and coordinate interactions between the two components in vivo. It has been well known that the elongation of microtubule is accomplished by addition of tubulin dimers to the growing end and suggested that specific regulatory factors are involved in this process. However, the detailed mechanism of the process in vivo is not understood yet. When microtubules grow in vivo, they must pass through (or interact with) the actin cytoskeleton which fills the cytosol of a cell. It suggested us the possible role of the actin cytoskeleton in the elongation of the cytoplasmic microtubules and, possibly, in the formation of the microtubule system.

To address these questions, the de novo formation of microtubule system during differentiation of a single cell eukaryote, *Naegleria gruberi* has been studied. When transferred from a growing medium into an aquatic environment, *N. gruberi* amoeba, which has actin cytoskeleton but no microtubules except the nuclear spindle fibers which are formed briefly during mitosis, undergoes a rapid and synchronous differentiation into a flagellate within 2 h (Fulton, 1977; Walsh, 1984). During this differentiation, *N. gruberi* sequentially forms micro-tubule based organelles de novo: basal bodies are formed at approximately 55 min after the initiation of differentiation, visible flagella at 70 min, and then cytoskeletal microtubules are assembled from the base of the growing flagella at 80 min. With the formation of this microtubule system, amorphous *N. gruberi* amoebae transform into flagellates with a regular contour and polarized distribution of cellular organelles. Using cytochalasin D and BDM which inhibit actin and myosin, the possible role of actin and myosin on the formation of microtubule system during differentiation of *N. gruberi* was investigated.