September 2000

Integrity of the actin cytoskeleton required for both phagocytosis and macropinocytosis in *Dictyostelium discoideum*

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Yuan, A. D. and C. P. Chia 2000.

Integrity of the actin cytoskeleton required for both phagocytosis and macropinocytosis in Dictyostelium discoideum. Session # 1955.

Published in Abstracts: 40th American Society for Cell Biology Annual Meeting, in *Molecular Biology of the Cell* **11** (supplement): pp. 376a377a.

1955

Integrity of the actin cytoskeleton required for both phagocytosis and macropinocytosis in Dictyostelium discoideum

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Filamentous (F-) actin is enriched in cellular extensions, such as phagocytic cups and macropinocytic crowns, of Dictyostelium discoideum amebae. Previous studies of actin-disrupting agents that implicated the involvement of the actin cytoskeleton in Dictyostelium phagocytosis and pinocytosis, however, have yielded conflicting results. We show that the integrity of the actin cytoskeleton is required for both phagocytosis and macropinocytosis in D. discoideum with latrunculin A (latA), which binds to monomeric actin, and cytochalasin A (cytA), which caps the plus end of actin filaments. Using rhodamine-phalloidin to visualize F-actin, cells treated for 30 min. with 1 to 4 μM of latA displayed an increasing dissolution of the cortical actin cytoskeleton that was accompanied by the appearance of numerous cytoplasmic dots of F-actin. In parallel, phagocytosis of fluorescently labeled yeast and macropinocytosis of the fluid-phase marker fluorescein isothiocyanate-dextran both were inhibited in a dose-dependent manner. Cells were nearly devoid of F-actin at latA concentrations greater than 5μM whereas the uniform distribution of monomeric actin appeared unaffected. Cells gradually recovered their intact actin cytoskeleton and concomitantly, their phagocytic and macropinocytic activities when latA was removed by washing. To achieve 50% inhibition of phagocytosis or macropinocytosis, five-fold more cytA than latA was required. Unlike latA-treated cells, cytA-treated cells stained with rhodamine phalloidin retained an actin cytoskeleton even at high concentrations (>25 μM), but were smaller and rounder than untreated cells. The cortical F-actin, however, appeared irregular, and almost discontinuous, which made the cells seem stiff and rigid in comparison to normal cells that looked more fluid and plastic. The distinctive alterations in the cytoskeletal patterns reflected the specific modes of action of the drugs on the actin network that was vital for both phagocytosis and macropinocytosis.