Immunofluorescence study on the intracellular digestion process of a microinjected mitochondria fraction in *Paramecium multimicronucleatum*

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SUMMARY

Autophagy is an ubiquitous process that occurs in all eukaryotic cells. During autophagy, cytosol and organelles are sequestered within double-membrane vesicles that deliver their contents to a lysosome/vacuole in which the macromolecules are degraded and recycled. Substantial progress has been made in identifying the proteins that are required for autophagy and in understanding its molecular basis; however, many questions remain unanswered. For example, neither the mechanism of vesicle formation nor the origin of the donor membrane is known. To study the mechanism of autophagic vacuole formation, we microinjected an isolated fraction of mitochondria of *Paramecium multimicronucleatum* into the cells of an identical clone. Prior to the microinjection, the isolated mitochondria fraction was stained with 5,5,6,6'-tetrachloro-1,1',3,3'-tetaethyl-benzimidazolylcarbocyanine iodide (JC-1) and was physically damaged by slow freezing. The isolated mitochondrial fraction was then analyzed for digestive vacuole (DV) membranes using three monoclonal antibody markers. We used mAb anti-B2 antigen, mAb anti-B3 antigen, or mAb anti-C5 antigen as the marker for membranes of discoidal vesicles/cytopharynx/DV-I, acidosome/DV-II, or all the DVs, respectively. Immunofluorescence studies of these mAb markers demonstrated that the membranes of vacuole-containing JC-1 were labeled with the three markers that were used. The injected mitochondoria fraction was therefore definitely delivered to the lysosomal system. This probably occurred because the mitochondrial fraction was recognized as exogenous material by the cell itself, even though the fraction was of endogenous origin.