

CELL FUSION IN THE CELLULAR SLIME MOLD,
DICTYOSTELIUM DISCOIDEUM

H. ONO, S. KOBAYASHI, and K. YANAGISAWA. From the Department of Biology, Tokyo Metropolitan University, Tokyo 158, Japan, and the Isotope Research Institute, Tokyo 158, Japan

INTRODUCTION

The myxamebae of the cellular slime mold feed upon bacteria and multiply by fission. As soon as the food supply is exhausted fission ceases. They aggregate and form migrating pseudoplasmodia, then fruit (Bonner, 1959).

Huffman and Olive (1964) reported that in *Dictyostelium discoideum* some cell fusions were observed at the very early aggregation stages, but they observed no fusion at later stages. Contrary to this finding, electron microscope analysis has revealed that cell fusions take place not only at the very early stages, but also at the late stages of development, such as migration and culmination of development, in *D. discoideum*.

MATERIALS AND METHODS

D. discoideum haploid strain NC-4 were used. The myxamebae were grown at 22°C on nutrient agar in association with *Aerobacter aerogenes* (Sussman, 1966), harvested, and suspended in cold distilled water at a concentration of 2×10^7 per ml. The suspension (0.5 ml) was taken and plated on a Millipore filter resting on an absorbent pad saturated with salts (Sussman and Lovgren, 1965). Samples of the various stages of the development were taken and fixed in a 5% aqueous, unbuffered solution of barium permanganate for 30 min at 0°C (Hohl and Hamamoto, 1969). Specimens were then dehydrated in ethanol, followed by propylene oxide, and embedded in Epon 812. The sections were stained with lead citrate followed by uranyl acetate, and observed with a JEM 100 U electron microscope.

RESULTS AND DISCUSSION

Cell fusions were observed between adjoining cells in migrating and culminating masses during

the development of *D. discoideum* (Fig. 1). The membranes between the two cells almost disappeared and a mitochondrion and a prespore vacuole were present in the cytoplasm in between. Prespore vacuoles were observed only in the cells located in the rear region of the migrating mass (Hohl and Hamamoto, 1969, Maeda and Takeuchi, 1969). Ashworth, Duncan, and Rowe (1970), and Kirk, Mckeen, and Smith (1971) reported the existence of small cytoplasmic bridges between the cells of *D. discoideum*. We also observed those figures relatively often at most stages of the development, but the figure of cell fusion found is clearly different from that of such cytoplasmic bridges. For instance, the width of cytoplasmic bridges is narrower than 0.4–0.5 μ in cell connections in most cases, but in cell fusions the connecting bridge is wider than 2.5 μ .

Huffman and Olive (1964) examined the cells with a light microscope and reported that most cell fusions took place at the stages of preaggregation and very early aggregation, and that fused cells were not observed once myxamebae enter aggregation streams. However, genetic evidence (Loomis and Ashworth, 1968; Sinha and Ashworth, 1969) has shown that cell fusions would take place also at the late stages of the development at the frequency of 1×10^{-3} to 1×10^{-4} . Our electron microscope observations indicate clearly that cell fusions take place also at the stages of migration and culmination of development.

Received for publication 10 March 1972, and in revised form 4 May 1972.

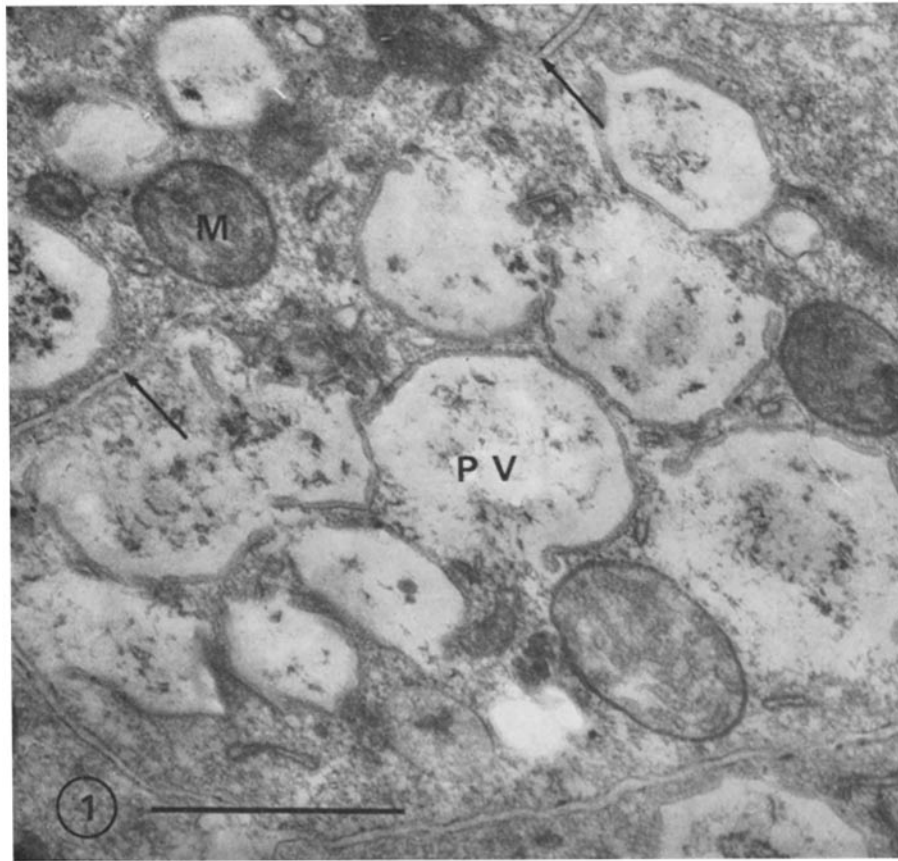


FIGURE 1 An extensive cytoplasmic bridge between adjoining cells in culminating mass of *D. discoideum*. The membrane between the two cells completely disappears (arrows), and a mitochondrion and a prespore vacuole are present in the cytoplasm in between. Prespore vacuoles appear specifically in prespore cells at the beginning of the migration stage and disappear right before spore formation. *M*, mitochondrion; *PV*, prespore vacuole. Scale line is $1 \mu \times 32,000$.

REFERENCES

- ASHWORTH, J. M., D. DUNCAN, and A. J. ROWE. 1970. Changes in fine structure during cell differentiation of the cellular slime mold *Dictyostelium discoideum*. *Exp. Cell Res.* **58**:73.
- BONNER, J. T. 1959. The cellular slime molds. Princeton University Press, Princeton.
- HOHL, H. R., and S. T. HAMAMOTO. 1969. Ultrastructure of spore differentiation in *Dictyostelium* the prespore vacuole. *J. Ultrastruct. Res.* **26**:442.
- HUFFMAN, D. M., and L. S. OLIVE. 1964. Engulfment and anastomosis in the cellular slime molds (Acrasiales). *Am. J. Bot.* **51**:465.
- KIRK, D., W. E. MCKEEN, and R. SMITH. 1971. Cytoplasmic connections between *Dictyostelium discoideum* cells. *Can. J. Bot.* **49**:19.
- LOOMIS, W. F., and J. M. ASHWORTH. 1968. Plaque-size mutants of the cellular slime mould *Dictyostelium discoideum*. *J. Gen. Microbiol.* **53**:181.
- MAEDA, Y., and I. TAKEUCHI. 1969. Cell differentiation and fine structures in the development of the cellular slime molds. *Dev. Growth Differ.* **11**:232.
- SINHA, U., and J. M. ASHWORTH. 1969. Evidence for the existence of elements of a para-sexual cycle in the cellular slime moulds, *Dictyostelium discoideum*. *Proc. R. Soc. Lond. B Biol. Sci.* **173**:531.
- SUSSMAN, M. 1966. *Methods Cell Physiol.* **2**:397.
- SUSSMAN, M., and N. LOVGREN. 1965. Preferential release of polysaccharide transferase during cellular differentiation in the slime mold, *Dictyostelium discoideum*. *Exp. Cell Res.* **38**:97.