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Scanning Electron Microscopy in Cell Biology and Medicine

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SCANNING ELECTRON MICROSCOPY IN CELL BIOLOGY AND MEDICINE

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May 11th-15th, 1980, Kyoto, Japan

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Preface

The morphologists researching the fine structure of organisms have always desired to observe more and more minute structures. The transmission electron microscope has to a large extent answered this desire and contributed to clarifying the fine structure of cells and tissues. We who are living in the three-dimensional world, however, have felt unsatisfied with the flat, plain images obtained from the transmission electron microscopy of ultra-thin sections.

To fulfill this need, the scanning electron microscope provides vivid three-dimensional images. In the beginning it was ridiculed as an expensive toy of morphologists because of its poor resolving power and unrefined techniques of specimen preparation. Gradually, however, the instruments and techniques have been advanced and scanning electron microscopy has become one of the most attractive and useful methodologies in morphological research. One after another, beautiful photomicrographs revealing the three-dimensional structures of cells and tissues have been rapidly accumulated by researchers in many biomedical fields.

Ten years have passed since the scanning electron microscope became useful in the biological field. The biological and medical researchers using scanning electron microscopes feel that they have arrived at a critical point from which fresh developments should be made. In addition to this, Japanese researchers and Japanese scanning electron microscopes in this decade have made major contributions to this field. Nevertheless Japanese scientists had not had sufficient chances to exchange their findings and ideas with over-sea researchers. These are the reasons why we held the international symposium, "Scanning Electron Microscopy in Cell Biology and Medicine" in Japan.

The meeting was held in Kyoto, May 11-15, 1980, on the occasion of the Aoi-festival, one of the most popular festivals in this old capital of Japan. Eighty of the most active researchers in the world participated in this symposium with vigorous scientific discussion and intimate personal communication. We believe that the symposium achieved its purpose which was to give a clear overview on the present day status of scanning electron microscope studies of cells and tissues in order to discover ways for advancement in the field. The papers that were shared were representatives of a variety of biomedical fields and contained many high level preparation techniques and unique applications of scanning electron microscopy. The results of the symposium have strengthened the view that ultrastructural research by scanning electron microscopy, critically evaluated, will open new avenues to biological and medical study.

This volume presents the proceedings of the symposium. It is our hope that this volume will encourage the researchers interdisciplinarily and internationally, and stimulate new developments in the applications of scanning electron micrscopy.

The symposium was supported financially by the Japan Society for the Promotion of Science, the Japan World Exposition Commemorative Fund, the Kajima Foundation, Eisai Co., Ltd., Hitachi Ltd., Nissei Sangyo Co., Ltd., Hitachi Koki Co., Ltd. and Mitsubishi Heavy Industrial Ltd. We express our cordial thanks for their generous support.

We thank Mr. T. Sayama and Mr. K. Imazu of the Eisai Co., Ltd. who helped us in management of the symposium and its associated social events. Cordial thanks are due to Miss Tomoko Kawakami for her enthusiastic co-operation in managing the symposium and in editing these proceedings.

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DYNAMICS OF CULTURED L CELLS AS STUDIED BY CINEMICROSCOPY AND SCANNING ELECTRON MICROSCOPY

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ABSTRACT

The dynamics of cultured L cells have been studied by phase contrast cinemicroscopy and scanning electron microscopy (SEM). The morphology and motility of an L cell greatly change through the cell cycle. During M the cell is spherical; during G1 fan-shaped; and during S and G2 polygonal. Microvilli and filopodia are greatest in number and length during M, decrease during G1, and increase again from S to G2. In contrast ruffles are prominently formed during G1, when the microvilli and filopodia decrease. Cell locomotion, closely related with the formation of ruffles, is most active during this period. Ruffles show wavy motion consisting of three stages, protrusion, standing up and retraction. Cytochalasin B stops the ruffling movement and the cell periphery assumes saw-tooth like edge. From the convex parts of the zigzag edge emerge microprojections, which move with the same periodicity and in a similar manner as the ruffles. On the other hand, colchicine treatment shifts the ruffling movement to the pseudopodial movement and diminishes the cell polarity. The roles of cytoplasmic fibers in the ruffling movement is discussed.

KEY WORDS cultured L cells / cell cycle / surface morphology / cell locomotion / ruffles / filopodia / microfilaments / microtubules / colchicine / cytochalasin B

Cultured cells on solid plane substratum have been observed by many light microscopists to show various movements. Cellular dynamics comprises division, shape changes, locomotion, etc. Intracellular dynamics includes movement of chromosomes, pinocytotic vesicles, pigment granules, mitochondria, etc.

Electron microscopically there are cytoplasmic fibers—microtubules, 10 nm-filaments, sheath-typed microfilaments, and lattice-typed microfilaments—which are considered to be closely related with the cell dynamics (12, 23, 46, 54).

The microtubules carry the chromosomes toward the both polar sites of the mitotic cell. They function as a cytoskeleton as well (8, 20). The 10 nm-filaments are associated with the migration of cytoplasmic organelles (13, 21). The sheath-typed microfilaments are involved with the cytoplasmic streamings. The lattice-typed microfilaments are considered to be directly responsible for the cytokinesis, ruffling movement, pinocytosis, etc. (23, 45, 53). Cytochalasin B and colchicine, which disorder the lattice-typed microfilaments and microtubules respectively, can provide excellent means for analyzing the roles of the cytoplasmic fibers in the cell dynamics.

This paper will deal with the functions of the cytoplasmic fibers in the movement of the ruffles of L cells. Preceding this subject a description of cell dynamics through the cell cycle will be given.

DYNAMICS OF L CELLS THROUGH CELL CYCLE

The cell cycle is divided into four periods: mitotic (M) period, 1st gap (G1) period, DNA synthetic (S) period, and 2nd gap (G2) period. L cells originating from the murine subcutaneous connective tissue, like many other cultured cells, have a cycle period of 23 hr, of which 1 hr is for M, 9 hr for G1, 8 hr for S, and 5 hr for G2. During the cycle cells undergo the synthesis, metabolism, and decomposition of materials (34). Just as such intracellular material changes are specific to each cycle period cell, surface morphology is characteristic to particular periods (37, 43). The dynamics and surface structures of L cells through the cycle have been observed by phase contrast cinemicroscopy and SEM.

In M period, an L cell takes a spherical form about 15 μm in diameter (Figs. 1a, b). On the cell surface two types of projections are distinguished. One are microvilli, about 0.1 μm in diameter and about 1 μm in length, covering the free surface of the cell. The other are filopodia, spiny projections 0.1-0.5 μm in diameter and 1-20 μm in length spreading radially from the lower lateral sides of the cell. The cell is attached with the tip ends of these filopodia and microvilli to the substratum. This spherical

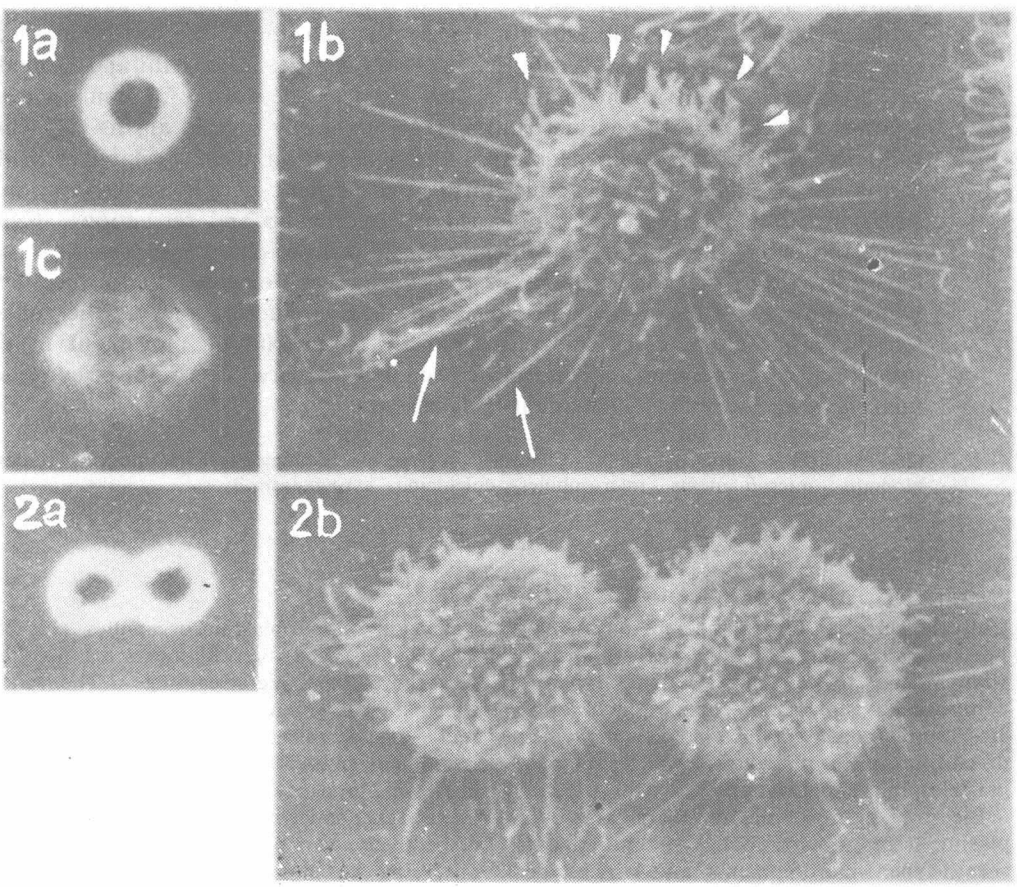

Fig. 1 **a.** Phase contrast micrograph of a living L cell in mitotic phase. ×800. **b.** Scanning electron micrograph of L cell comparable to that in Fig. 1a. The upper surface of the cell is covered with many long microvilli (arrowheads). There are filopodia (arrows) projected radially from the lower lateral region of the cell. ×1800. **c.** Fluorescent micrograph of the mitotic L cell after treatment with antibody against tubulin. Microtubules found in the mitotic spindles. ×1,500. (Fig. 1a, 3a, 6a courtesy of Dr. Yahara) Fig. 2 **a.** Phase contrast micrograph of two daughter L cells immediately after cell division (G1 period). ×800. **b.** Scanning electron micrograph of the cells comparable to that in Fig. 2a. ×2,500