Ultrastructure of the Circumferential Microfilament Bundle and its Role in Maintaining Cell Shape in Cultured Retinal Pigmented Epithelial Cells

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ABSTRACT

Dedifferentiated pigmented epithelial cells exhibit a bipotential phenotype when cultured. Expression of either of two differentiated phenotypes (pigmented or lentoid) is effected by signals in the culture environment which are mediated by the cell surface and its associated cytoskeleton. This paper describes a structural analysis of the circumferential microfilament bundle with the cell membrane of cultured pigmented epithelial cells from chick retina. Techniques of electron microscopy reveal that microfilaments associate both with zonula occludens junctions and with membrane domains which are separate from specialized junctions. At both sites, intermembrane linkages are present which provide structural continuity between cells at their apical surfaces. This ultrastructure contributes to the anchoring of the circumferential bundle to the cell membrane, which in turn provides a structural basis for the generation of tensile force and the characteristic polygonal shape of differentiated cells in retinal pigmented epithelium.

KEYWORDS

Circumferential microfilament bundle; cell membrane; cell junction; cell contact; retinal pigmented epithelium.

INTRODUCTION

The differentiated pigmented epithelial cell is characterized by presence of melanin granules, cuboidal shape and a distinctive circumferential microfilament bundle (CMB) at the apical compartment of each cell [1-6]. This paper reports a description of the structure of the CMB and its attachment to the lateral cell membrane of cultured pigmented epithelial cells.

Since Owaribe and Masuda [7] demonstrated the contractile ability of circumferential microfilament bundles isolated from chick retinal pigmented epithelium, one can postulate that (a) force must be transferred from the CMB to the cell membrane by attachment of microfilaments to the cell membrane and that (b), to achieve sufficient rigidity for maintenance of polygonal shape, microfilaments must be bundled together. Furthermore, such a model predicts that microfilament bundles must be linked (anchored) on their sides to the cell membrane and that force is transduced between cells by junctional specializations (e.g. zonula occludens and adherens).

This paper presents information on the structural nature of the cell membrane and its internal organization which corresponds to attachment to the circumferential microfilament bundle in differentiated pigmented epithelial cells from 8 day chick embryos.

Techniques of electron microscopy permit visualization of the CMB with zonular junctions in the apical cell compartment and with membrane domains which are not components of zonules. Intermembrane linkages between cells at the apical cell compartment are also described. This ultrastructure contributes to the anchoring of the CMB to the cell membrane, which in turn provides a structural basis for the generation of tensible force and the maintenance of differentiated, polygonal cell shape.

MATHERIALS AND METHODS

Cell Culture

Cultures of retinal pigmented epithelial cells were prepared from eyes of 8 d chick embryos by the techniques described by Eguchi and Okada [7] and maintained either in culture dishes or on plastic cover slips.

Electron Microscopy

For scanning electron microscopy, cultures were rinsed in phosphate buffered saline and fixed by immersion in 2.0% glutaraldehyde in 0.1 M cacodylate buffer (1 h; pH 7.4; room temperature). Prior to fixation, some coverslips were immersed for 30 sec to 1 min in an extraction buffer containing .5% Triton X-100; 4 mM glycerin; 10 mM PIPES; 2 mM Mgcl2; 50 mM KCl; and 2 mM EGTA (pH 7.4, room temperature) to remove cell membranes and permit visualization of the detergent insoluble cytoskeleton [8]. All specimens were rinsed folloving fixation, dehydrated through a graded ethanol series and dried by the critical point method. Cells were coated with Au-Pd alloy and examined in an Hitachi S-800 scanning electron microscope.

Freeze-fracturing and Deep Etching

Specimens prepared for freeze-fracturing and deep-etching [9] were lightly fixed in 1.0% glutaraldehyde in 0.1 M sodium phosphate buffer (10 min; pH 7.4; room temperature), rinsed in buffer at room temperature, immersed in distilled water on ice, and transferred to 15% methanol in distilled water (4°C). Regions of the monolayer selected for maximum expression of the differentiated cell phenotype were picked up on gold stubs which were then placed on the tip of a spring-loaded plunger compatible with a "Slammer" freezing chamber. The tip of the plunger was rapidly pressed against he surface of a polished copper-gold alloy plate cooled with liquid helium. Individual specimens were fractured at -196°C at 2-5 X 10^{-7} Torr (removing approximately 2.0 µm of ice); and rotary shadowed (60 rpm) with platinum-carbon at a 24° angle. The replica was stabilized with a 3.5-5.0 nm layer of carbon evaporated at 90° perpendicular to the surface of the specimen, cleaned with methanol and bleach and examined in a JEOL 1200 EX transmission electron microscope [11].

RESULTS

Pigmented epithelial cells from colonies on substrates approximately 10 d following seeding of either primary or secondary cultures. Each colony exhibits a gradient of differentiated states. Cells at the perphery are "undifferentiated" and exhibit heteromorphic shapes characteristic of motile cells. In contrast, cells in the center of each colony exhibit polygonal shapes and pigment granules characteristic of the differentiated phenotype. The scanning electron microscope (Fig. 1) shows individual differentiated pigmented epithelial cells to have an apical surface characterized by numerous small projections and a smooth perimeter where contacts with adjacent cells are established. When the cell membrane is removed following extraction with Triton X-100 (Fig. 2), it is apparent that this apical compartment is occupied by a bundle of microfilaments (6 nm in diameter) which borders the circumference of each cell. Each polygonal ring of microfilaments abuts the cell membrane on its outer boundary and intersects the 10 nm (intermediate) filament component of the cytoskeleton on its cytoplasmic boundary.

The cicumferential microfilament bundle excludes large cytoplasmic structure, whereas the cell nucleus, membranous organelles and pigment granules are supported by the remainder of the cytoskeleton. In addition, the CMB separates an apical cytoplasm from the central and basal cytoplasm.

Freeze-fracturing through the apical compartment confirms the presence of zonular junctions (Fig. 3) and further reveals the complex organization within the cell membrane adjacent to the CMB. Discontinuous strands of intramembrane particles (resembling focal tight junctions) are oriented in the apical-basal plane of the cell membrane. The vertical particle arrays (seen in the P-face in Fig. 3) intersect with horizontal depressions observed in the E-face of the micrograph.

In addition, the technique of deep etching produces a useful, three-dimensional image (Fig. 4) which shows the CMB inmmediately adjacent to the cell membrane. The cell membrane is seen as two closely apposed units linked together by intermembrane structures approximately 15 nm in length. The majority of 6 nm filaments in the CMB form a network which encompasses the boundary of the cell. Additional structures (lateral links) connect individual filaments (and

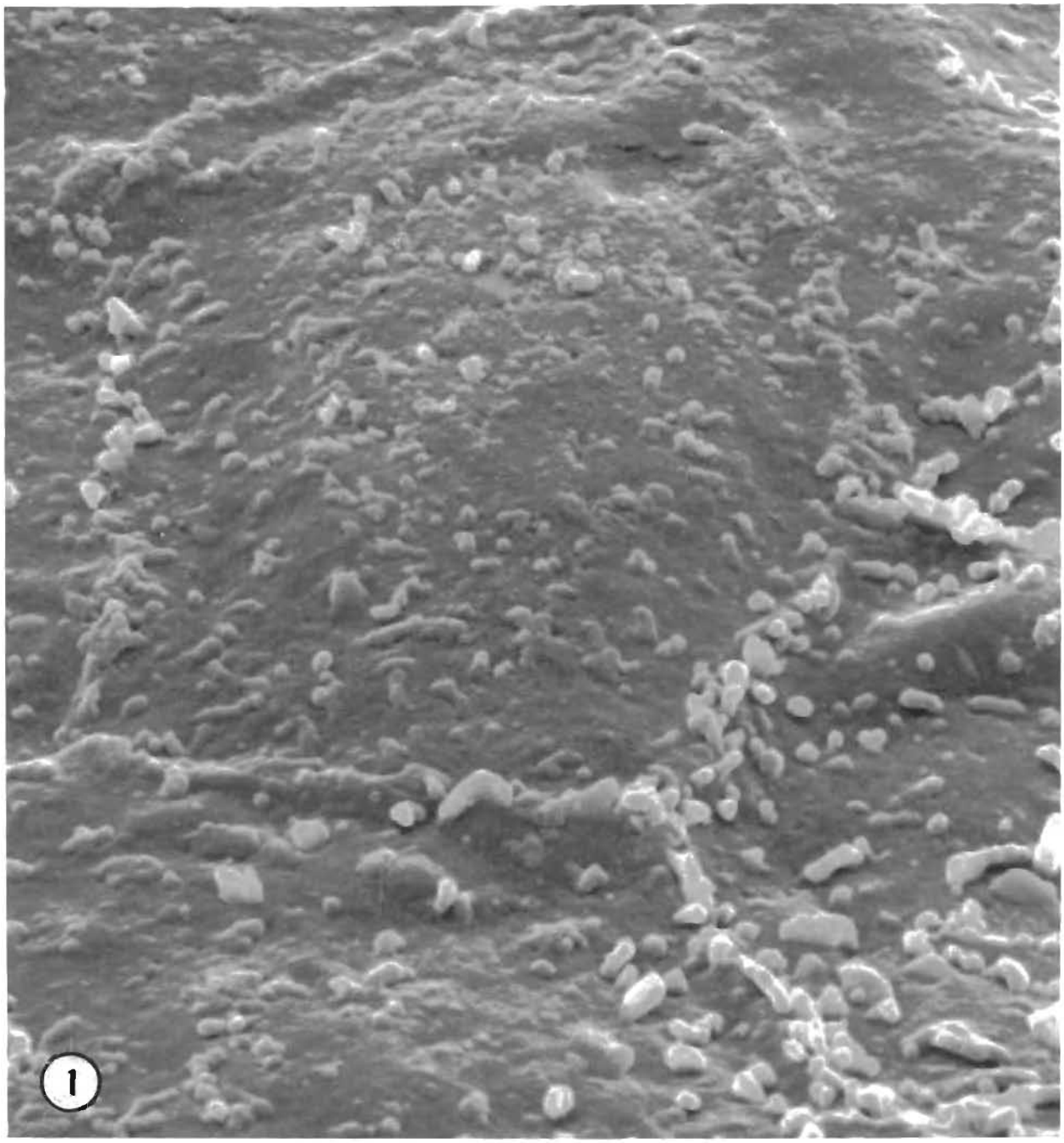

Fig. 1. Scanning electron micrograph of the apical surface of a cultured retinal pigmented epithelial (RPE) cell from an 8 d chick embryo. Note close association between cells and the polygonal shape of contacting cells. The circumferential microfilament bundle lies immediately beneath the apical surface at the outermost perimeter of each cell. X 2,000

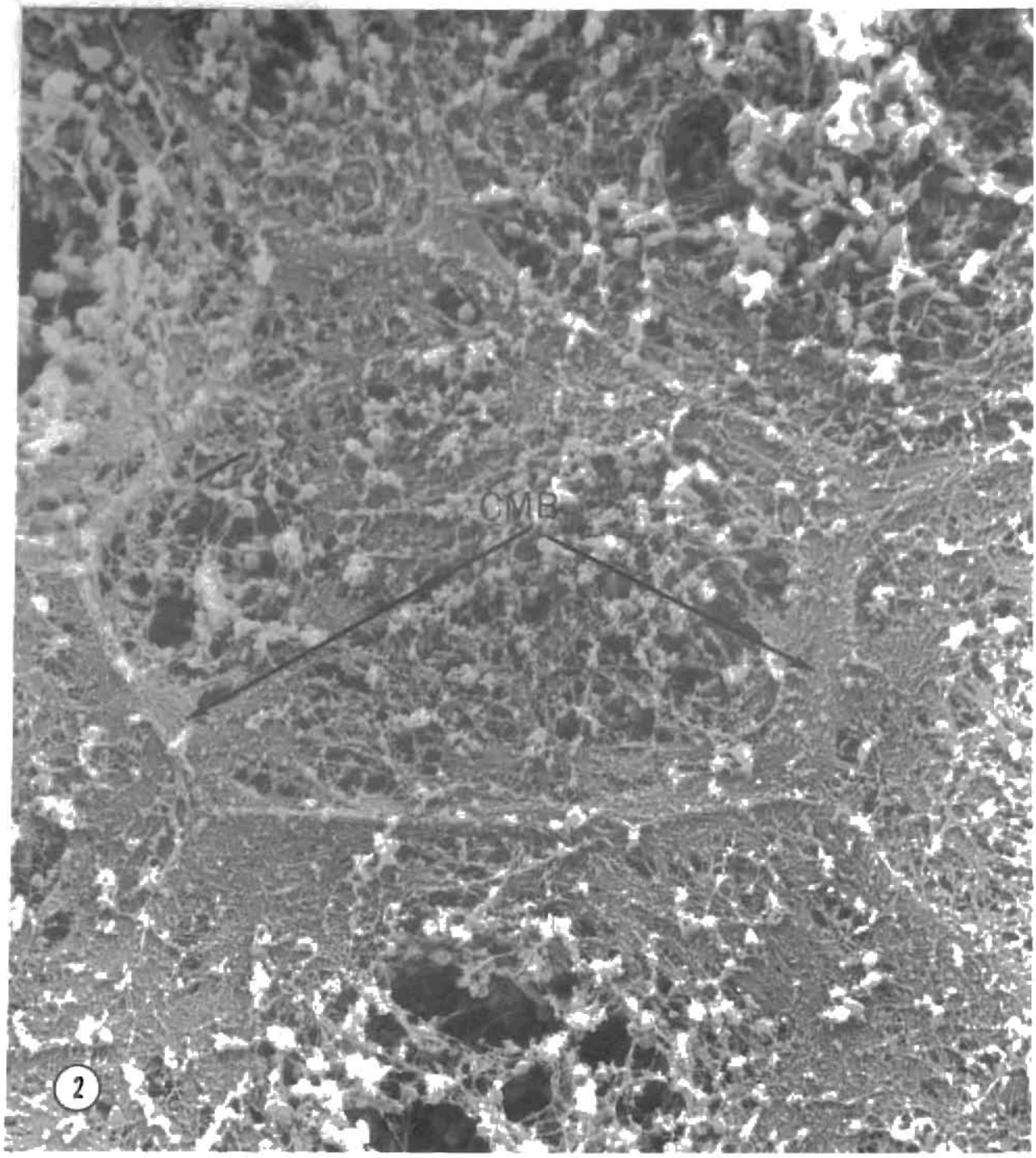

Fig. 2 Scanning electron micrograph of cultured RPE cell following treatment with Triton X-100 to reveal the circumferential microfilament bundle (CMB) and deeper intermediate filaments of the cytoskeleton. Small, ovoid structure are pigment granules. X 2,000

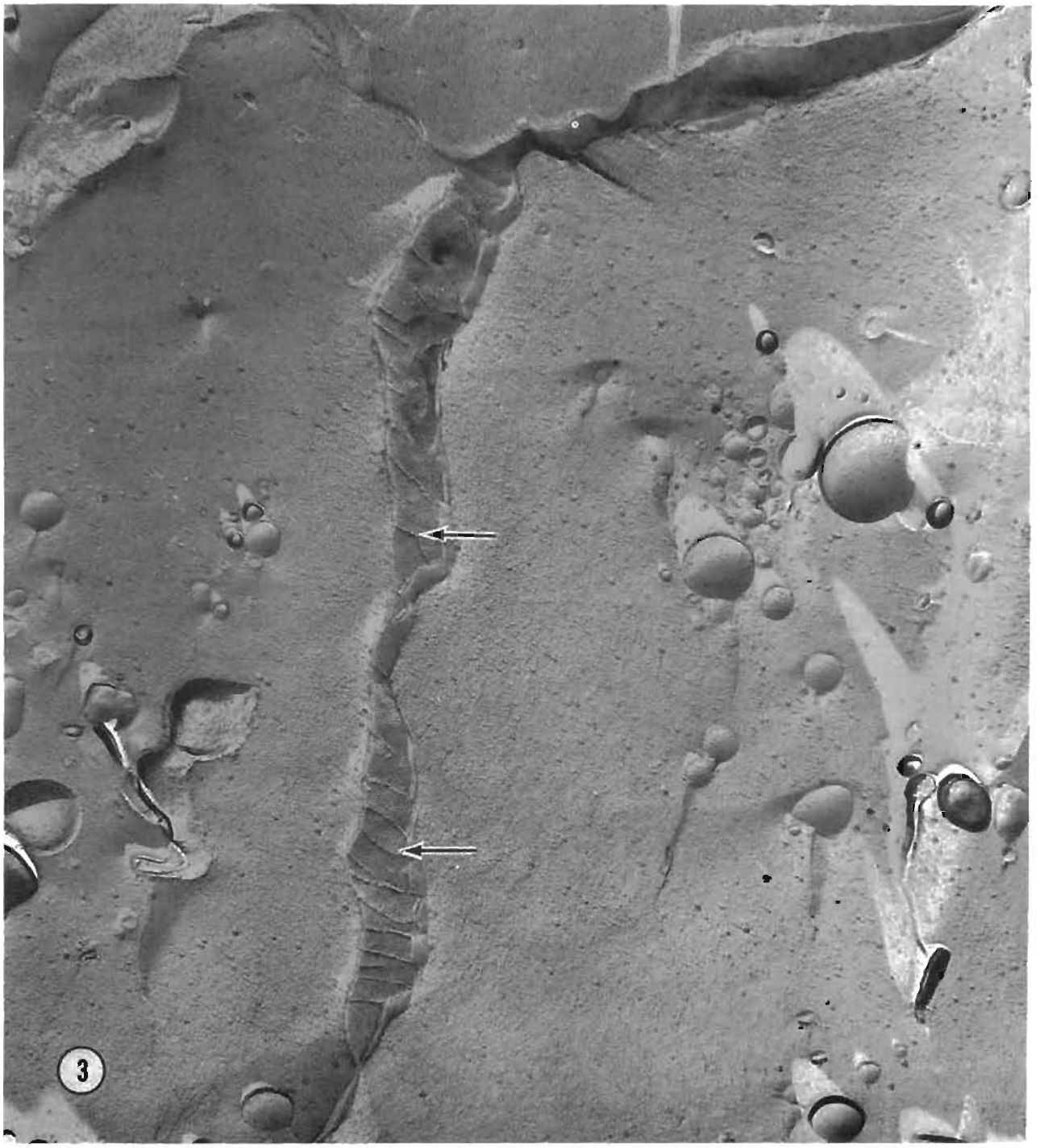

Fig. 3 Transmission electron micrograph of cultured RPE cells freeze-fractured through the apical compartment. Intramembrane particles (arrows) in the P face of the cell on the left are part of a zonular junction which is associated with the circumferential microfilament bundle in the apical compartment of the cell. X 30,000

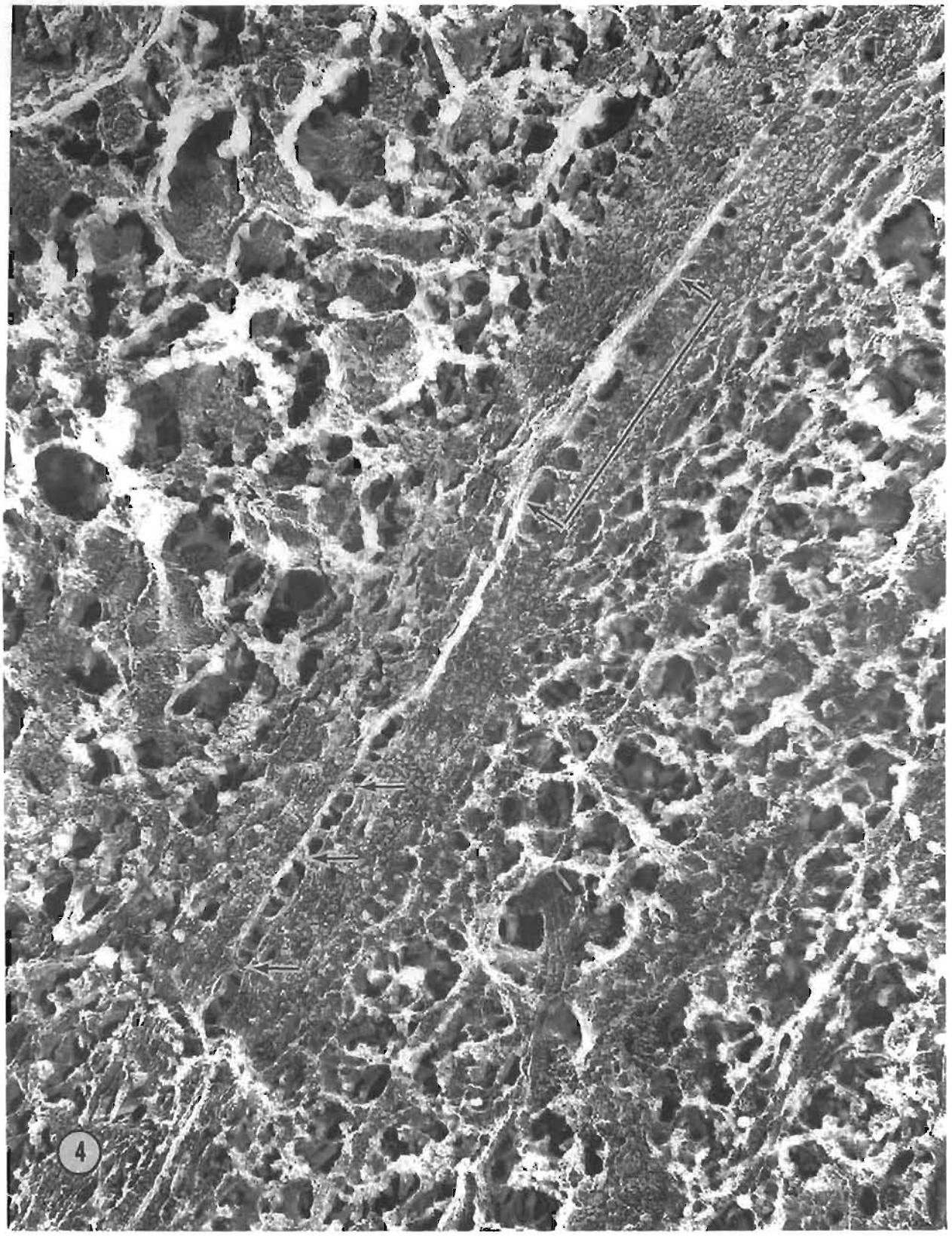

Fig. 4 Transmission electron micrograph of cultured RPE cells deep-etched following freeze-fracture. The CMB is clearly associated with zonular structures which are connected by intermembrane linkage elements (arrows). In addition, lateral linking structures are present between the CMB and adjacent cell membrane (bracket). X 30,000

presumably their associated binding proteins) to the cytoplasmic surface of the cell membrane. These lateral links are most prevalent along the sides of the CMB, in contrast to the corners where three cell boundaries converge. In these corner regions, deep etching reveals groups of actin filaments which terminate on the cytoplasmic surface of the cell membrane. The organization and composition of the cytoskeleton at the cell perimenter immediately beneath the CMB resembles a lattice of intermediate filaments with little, if any, organized bundles of microfilaments.

DISCUSSION

Crawford [1, 11] was among the first to demonstrate the ultrastructure of the apical compartment in cloned retinal pigmented epithelial cells. He speculated that the organization of the CMB and cellular microtubules were correlated with maintenance of cellular shape and function (phagocytosis of outer segments of the neural retina). In addition, he suggested that cell functions were controlled to some extent by the contacts and junctions formed between neighboring cells and their substrates. Furthermore, he speculated that a chronic contraction of microfilaments was characteristic of cells in the differentiated center of pigmented cell clones and that this condition maintained, in part, the differentiated phynotype of the pigmented cell.

Experimental demonstration of contractility in the circumferential microfilament bundle was reported by Owaribe, Kodama and Eguchi [3]. These investigators suggested that contractile properties of the CMB contributed to the morphogenetic cell shape changes evident in both transdifferentiation (in vitro) and during normal in vivo differentiation. It is important to note that contractility of the CMB requires Mg-ATP; does not require Ca^{++}; and is inhibited by the S1 subfragment of myosin. The latter observations were confirmed by Owaribe and Masuda [2] following successful isolation of CMB's from chick retinal pigmented epithelium.

In order to provide a structural basis for contractility, one must predict that the actin microfilaments in the CMB are (a) attached to the cell membrane, (b) bundled together to achieve sufficient rigidity to maintain polygonal shape, and (c) linked (anchored) on their sides and at their termini with the cell membrane to permit transduction of force between cells via junctional specializations. From the observations presented in this paper, it is clear that structural links develop between bundles of actin microfilaments in the CMB and the lateral cell membrane. These lateral links provide evidence for the attachment, via linking proteins, of microfilaments to the cytoplasmic surface of the plasmalemma. In addition, the observation of intercellular link structures provides a further structural basis for the transduction of contractile force between cells. Further investigation may elucidate the molecular nature of these linking elements and perhaps clarify further the nature of cell-cell attachment between cells in retinal pigmented epithelium.

RESUMEN

Células epiteliales pigmentadas diferenciadas, exhiben un fenotipo bipotencial cuando son cultivadas. La expresión de uno de los dos fenotipos diferenciados (pigmentado o lentoide) esta condicionada por señales en el microambiente del cultivo, las cuales, son mediadas por la superficie celular y su citoesqueleto asociado. Este trabajo describe un análisis de interelaciones estructurales del haz de microfilamentos circunferenciales con la membrana de células epiteliales pigmentadas de retina de pollo cultivadas. Técnicas de microscopía electrónica revelan que los microfilamentos se asocian con las uniones **zonula occludens** y con la membrana, las cuales, están separadas por uniones especializadas. En ambos sitios, están presentes uniones intermembrana, las cuales, proveen continuidad estructural entre las células y sus superficies apicales. Esta ultraestructura contribuye al anclaje del haz circunferencial a la membrana celular, la cual a su vez, provee una base estructural para la generación de fuerza tensil y la forma poligonal característica de las células diferenciadas en el epitelio retinal pigmentado.

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