Cell-Adhesion Molecule Uvomorulin Is Localized in the Intermediate Junctions of Adult Intestinal Epithelial Cells

K. BOLLER, D. VESTWEBER, and R. KEMLER
Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstr. 37-39, D-7400 Tübingen, Federal Republic of Germany

ABSTRACT Uvomorulin is a cell-adhesion molecule implicated in the compaction process of mouse preimplantation embryos and the aggregation of embryonal carcinoma cells. A rabbit antiserum against purified uvomorulin also reacts with epithelial cells of various adult tissues. In this study, we investigated the localization of uvomorulin on adult intestinal epithelial cells using electron microscopic analyses. Uvomorulin was shown to exhibit a highly restricted localization in the intermediate junctions of these cells. The results are discussed with respect to a possible adhesive function of uvomorulin on intestinal epithelial cells.

Compaction of preimplantation mouse embryos is the first morphogenetic event in embryonic development and is thought to be a prerequisite for the first differentiation step, the creation of trophectoderm and inner cell mass. During compaction, the formerly loosely adhering cells are joined tightly together, a process that is accompanied by the appearance of intercellular junctions on the outer cells of compact morulae (2).

Uvomorulin (UM)[1] is a cell-surface molecule that is involved in the compaction of preimplantation mouse embryos and the calcium-dependent aggregation of embryonal carcinoma (EC) cells. The identification of UM is achieved by producing rabbit anti-EC antibodies that interfere with the compaction of preimplantation embryos and the aggregation of EC cells (9). Purification of a molecule that reverses the antibody effect lead to an 84,000-mol-wt glycoprotein, a trypsin fragment of UM (6). A rat monoclonal antibody against the trypsin fragment (7) shows that the native UM molecule is a 120,000-mol-wt glycoprotein (12).

Uvomorulin has also been identified using a different experimental approach. When plasma membrane preparations of iodinated cells from an EC cell line and a differentiated cell line were compared by two-dimensional gel electrophoretic analysis, three glycoproteins (with approximate molecular weights of 120,000, 102,000, and 84,000) were present on EC cells but not on cells of the differentiated cell line (16). Rabbit antisera were raised against each of these spots cut out from the gels. By immunoprecipitation and immunoblotting analysis, it was shown that the lower molecular weight proteins are degradation products of the 120,000-mol-wt glycoprotein, which is identical to UM (16).

[1] *Abbreviations used in this paper:* EC, embryonal carcinoma; PBS, phosphate-buffered saline; UM, uvomorulin.

Distribution studies with rabbit anti-UM sera show that UM is present on all stages of preimplantation embryos. Later during embryonic development and in adult tissues, positive reaction is exclusively found on epithelial cells independent of their germ layer origin (16). In contrast to EC cells in which UM is uniformly distributed over the cell membrane (8), our studies showed that on adult epithelial cells, UM exhibits a highly restricted location. We discuss these results with respect to a possible functional role of UM on adult epithelial cells.

MATERIALS AND METHODS

Anti-UM Sera: The production and characterization of rabbit anti-UM sera has been described (16). Briefly, anti-UM antisera decompact cultured EC cells, PCC4azal, up to 1/600 dilution. Their Fab fragments inhibit compaction of preimplantation embryos and the aggregation of isolated PCC4azal cells. On EC cells, anti-UM gives positive staining up to 1/300 dilutions in an indirect immunofluorescence test. In immunoprecipitation and immunoblot experiments, all anti-UM sera recognize a pattern of glycoproteins (with molecular weights of 120,000, 102,000, and 92,000, and, more weakly, 84,000). For experiments described in this report antiserum raised against GP 120 was used.

Immunoblot: For immunoblot experiments, EC cell line PCC4azal was cultured in Dulbecco's modified Eagle's medium, 15% fetal calf serum in a 10% CO_2 atmosphere. Cells were lysed in 0.5% Nonidet P-40, 0.5% Triton X-100 in phosphate-buffered saline (PBS) [pH 7.2], and 1 mM phenylmethylsulfonyl fluoride for 30 min at 4°C. 50 μl of lysate (an equivalent of 10^6 cells) were put in each well of the polyacrylamide gel. Adult mouse small intestine (~15 cm) was washed in Ca^{2+}- and Mg^{2+}-free PBS and subsequently rinsed in 400 μl of SDS PAGE sample buffer; 120 μl were put in each well of the polyacrylamide gel. Proteins were separated by 10% SDS polyacrylamide electrophoresis (10) and transferred electrophoretically onto nitrocellulose sheets (Schleicher & Schüll, Inc., Keene, NH) as described (16). Bound anti-UM antibodies were detected with ^{125}I-labeled protein A (specific activity: 17 μCi per μg protein A).

Immunofluorescence: For indirect immunofluorescence tests, tissues of adult 129/Sv mice or Lou rats were fixed in 2% formaldehyde in PBS (pH 7.2) for 2 h at 4°C. The tissues were washed in PBS and frozen in 2.3 M sucrose. Cryostat sections (1–2 μm) were fixed for 10 min in cold methanol (−20°C), and washed in PBS; bound anti-UM antibodies were revealed with a

rhodamine-conjugated swine IgG fraction anti-rabbit IgG (Cappel Laboratories, Cochranville, PA) as described earlier (16). Preimmune serum was used on control sections.

Electron Microscopy: Tissues were Epon-embedded following the standard procedure as described by Luft (11). For the immunoelectron microscopic localization of anti-UM antibodies, tissues were treated in two ways.

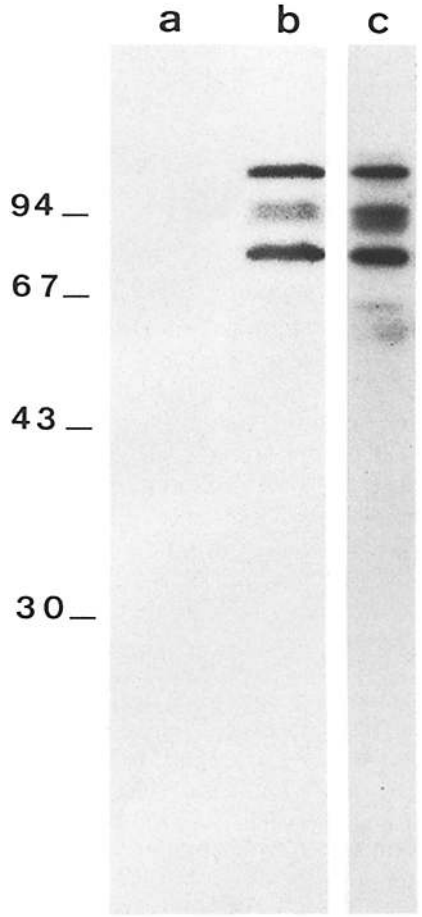

FIGURE 1 Immunoblots of cell lysates from mouse intestinal epithelial cells (lanes *a* and *b*) and EC cells (lane c). Bound antibodies were detected autoradiographically with [^{125}I]protein A. (Lane *a*) Preimmune serum; (Lanes *b* and c) anti-UM serum.

ULTRACRYOTOMY: Tissues were fixed in 2% freshly prepared formaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. Small blocks of tissue were immersed in 1.6 M sucrose in 0.1 M phosphate buffer overnight, frozen in liquid nitrogen, and mounted on an ultramicrotome with cryo-equipment (Sorvall MT-2B [Beckman Instruments, Inc., Palo Alto, CA]). Sectioning and the handling of the 100-nm sections were done as described (15), anti-UM and preimmune sera were used at 1/100 dilution, and antibody binding was revealed with protein A–labeled colloidal gold (7 or 12 nm) (14). After immunolabeling, sections were stained with 2% neutral uranyl-acetate and embedded in 1% methocoel containing 0.02% uranyl-acetate as described (3).

PLASTIC EMBEDDING: Tissues were fixed as described above and cut in small pieces. Dehydration and low-temperature embedding in Lowicryl K4M was carried out as previously described (13). After UV-polymerization, the blocks were dried overnight in a vacuum over a phosphopentoxide atmosphere that improved the subsequent sectioning. Sections (60 nm) were prepared on a microtome (LKB Ultrotome IV [LKB Instruments, Inc., Gaithersburg, MD]) and incubated in a solution of lysine, glycine, and ammonium chloride (0.02 M each) to minimize unspecific antibody reaction. Anti-UM and preimmune sera were used at 1/30 dilution followed by a protein A–colloidal gold incubation as described above. Sections were stained with 1% uranylacetate for 15 min and 1% lead acetate for 3–5 min. All preparations were examined in a Siemens Elmiskop Ia at 80 kV high voltage and photos were taken using Agfa Scientia films.

RESULTS AND DISCUSSION

Although the mechanism and function of cell-adhesion molecules is beginning to be understood in some systems (4), little is known about UM. Questions about the functional domain on the UM molecule or a presumable receptor for UM remain elusive. It is presently known that UM is a 120,000-mol-wt glycoprotein involved in the compaction of preimplantation embryos and the aggregation of EC cells. Given the finding that UM separates into the aqueous phase of Triton X-114 cell extracts it does not seem to be an integral membrane protein. Also, anti-UM sera react with adult epithelial cells independent of their germ layer origin (12, 16). The latter point suggests that one might learn more about UM if it was also studied on adult epithelial cells. Such an investigation is the focus of this report.

Polyclonal anti-UM antibodies recognize an identical pattern of proteins on immunoblots of small intestinal epithelial cells and on EC cells (Fig. 1). This structural resemblance of UM on both cell types suggests that UM might play a similar functional role for adult epithelial cells. Since a functional

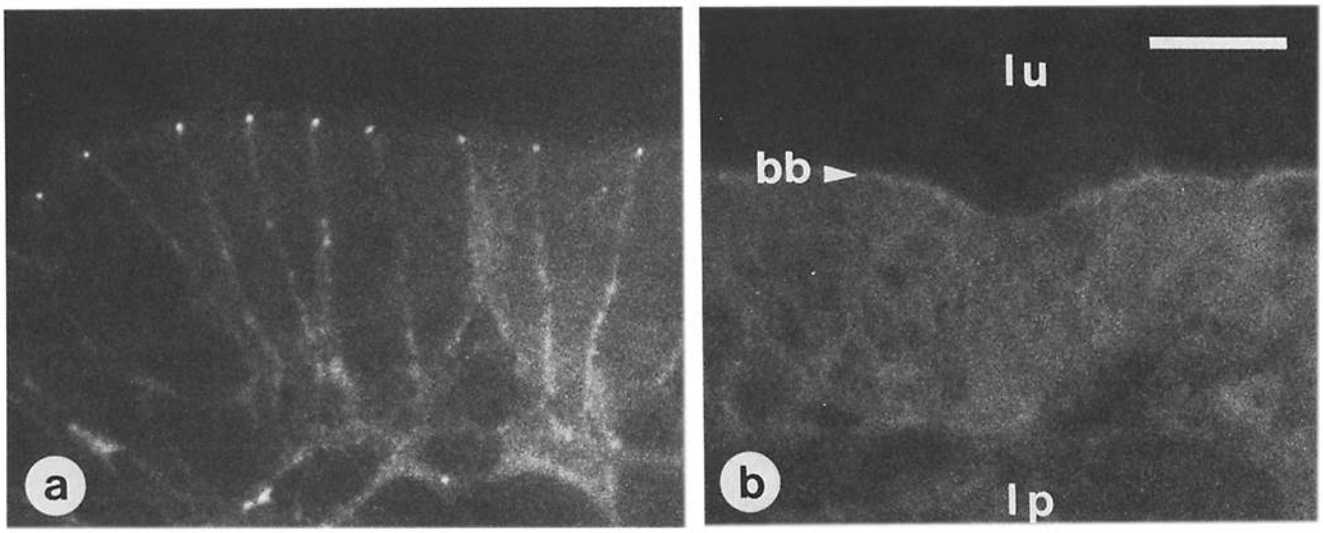

FIGURE 2 Indirect immunofluorescence test on cryostat sections of mouse small intestine. The cross-section of a villus shows strong staining in the junctional complex area of the epithelial cells with anti-UM (*a*) and preimmune serum (*b*). *lu*, Lumen; *lp*, lamina propria; *bb*, brush border. Bar, 20 μm. × 900.

aggregation test with intestinal cells would be difficult to perform, it was first attempted to obtain additional information by immunocytochemical and ultrastructural analysis. Anti-UM antibodies exhibit a rather restricted staining on adult epithelial cells. In indirect immunofluorescence tests, anti-UM staining was most prominent in the junctional complex area of cells while the remaining basolateral membranes were more weakly labeled (Fig. 2). The microvilli-containing apical membrane was always found negative. This restricted UM localization could be pinpointed to the intermediate junctions by immunoelectron microscopic analysis (Figs. 3 and 4). At this level, anti-UM reaction was almost exclusively localized in the intermediate junctions. No specific staining was observed in the tight junctions or desmosomes (Fig. 4). Preimmune serum was always negative for the junctional complex (Fig. 3*c*). However, in some preparations of mouse epithelial cells, staining of the microvilli was observed. This staining was taken as unspecific since it appeared equally with

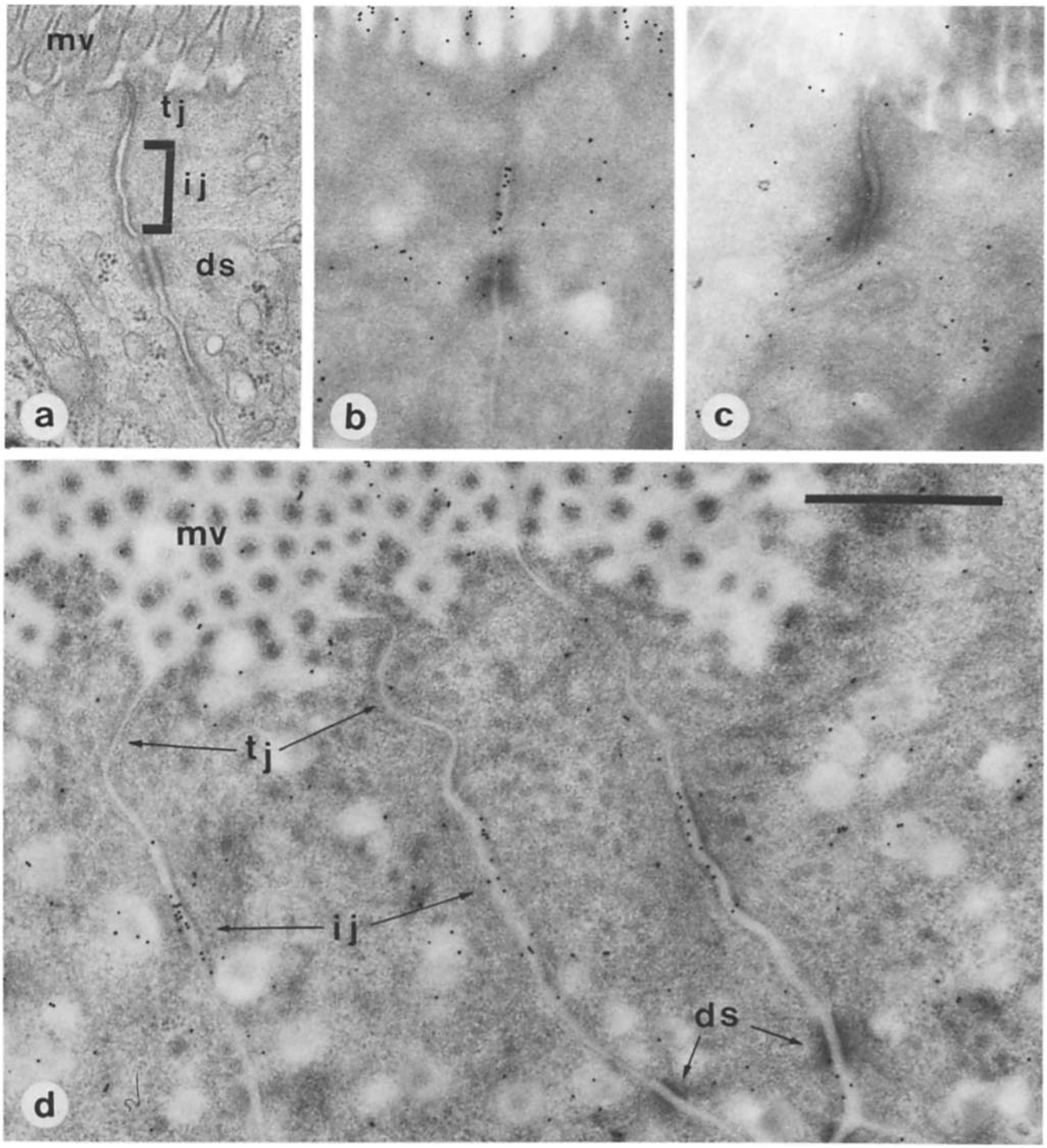

FIGURE 3 Electron micrographs showing immunolocalization of UM on mouse intestinal epithelial cells. (a) Section through the junctional complex of Epon-embedded mouse intestine. (*b* and *c*) Ultrathin frozen sections with anti-UM and preimmune serum, respectively. (*d*) Lowicryl-embedded tissue stained with anti-UM. Anti-UM staining is restricted to the intermediate junctions. Colloidal gold particles = 12 nm. *mv*, Microvilli; *tj*, tight junctions; *ij*, intermediate junctions; *ds*, desmosome. Bar, 1 μm. ×32,000.

preimmune and anti-UM serum and only occasionally in preparations of mouse but never of rat epithelial cells. Also, as shown above in immunofluorescence tests (Fig. 2), the apical membrane of epithelial cells was never labeled with anti-UM. Comparison of ultracryotomy and plastic embedding techniques gave similar results for mouse (Fig. 3, *b* and *d*) and rat (Fig. 5, *a* and *b*) epithelial cells; the most prominent anti-UM reaction was always found in the intermediate junctions. The anti-UM staining appeared to be between neighboring cells in both the mouse and rat intestinal epithelia. The ultrastructural localization of UM is consistent with the results of the Triton X-114 experiments mentioned above that suggest that UM is not an integral membrane protein. However, these data do not completely rule out the alternative possibility. The anti-UM staining of the basolateral membranes was always found stronger in immunofluorescence tests than in electron microscopical preparations. Whether this difference is due to different procedures or to differences in sensitivity remains an open question. However, the restricted localization of UM in the intermediate junctions compared with the basolateral membranes was significant in both techniques. To verify the differential localization of UM in the intermediate junctions and adjacent lateral membranes, we counted the concentration of gold particles per micrometer of membrane in seven mouse and four rat cells in which both membrane segments were present in the same photograph.

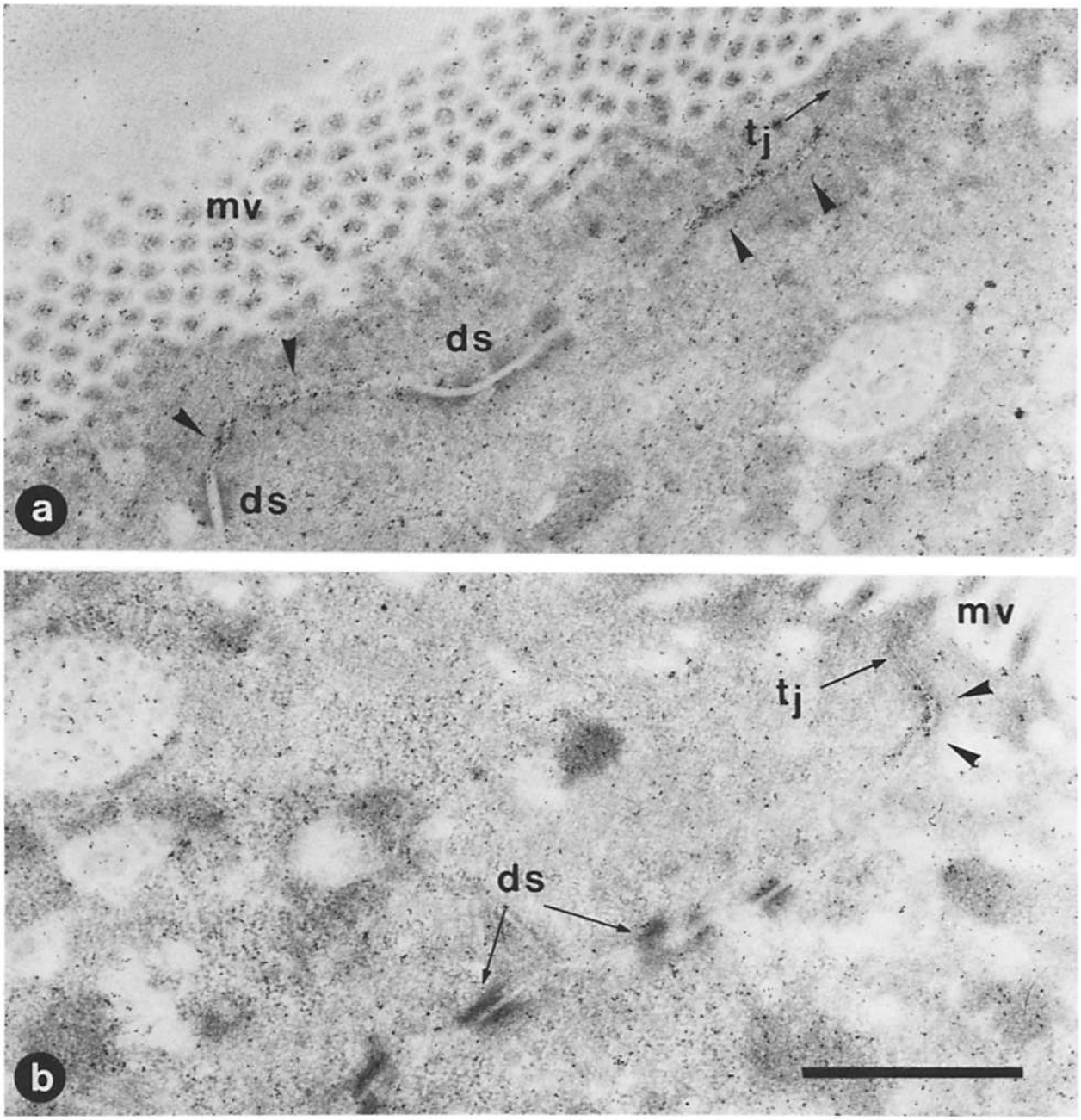

FIGURE 4 Electron micrographs of the junctional complex area of mouse intestinal epithelial cells from Lowicryl-embedded tissue. Anti-UM staining is localized in the intermediate junctions (arrowheads); no specific staining is observed in tight junctions (*tj*) or desmosomes (*ds*). (*a*) Sections through the junctional complex approximately parallel to the apical surface of the cell. (*b*) Sections through the junctional complex approximately parallel to the lateral surface of the cell. Colloidal gold particles = 7 nm. *mv*, Microvilli. Bar, 1 μm. × 33,000.

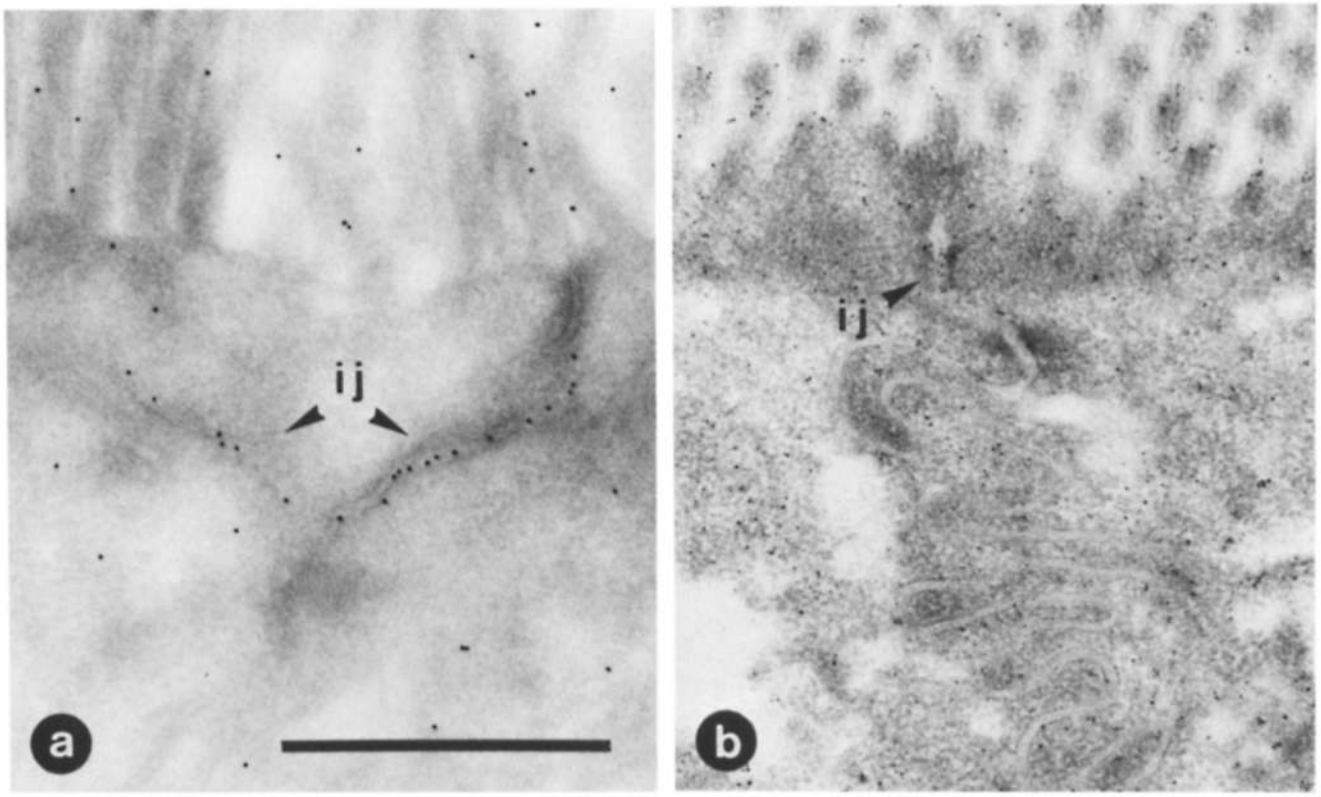

FIGURE 5 Electron micrographs showing immunolocalization of UM on rat intestinal epithelial cells of ultrathin frozen sections (*a*) and Lowicryl-embedded tissue (*b*). In *b*, compare the concentrated UM localization in the intermediate junctions with the adjacent lateral membranes. Colloidal gold particles = 12 nm in *a* and 7 nm in *b*. *ij* (arrowheads), Intermediate junctions. Bar, 1 μm. × 42,000.

The ratio of gold particles per micrometer of membrane between intermediate junctions and lateral membrane was 11 ± 2 and 31 ± 15 (SEM) for the mouse and rat cells, respectively. The difference between the two was due to noticeably less background in the rat sections rather than to more gold particles per unit length of intermediate junction membrane.

As a result of their physiological function, the intestinal epithelial cells have a unique form of motility that involves the terminal web and the corresponding junctional cell contact sides (1). It has been proposed that the motility in the brush-border region of intestinal cells is of circumferential constriction mediated by a contractile ring of microfilaments. Ultrastructural analysis revealed that this radial constriction occurs at the level of the intermediate junctions (1, 5). The predominant localization of cell-adhesion molecule UM in the intermediate junctions suggests that UM might play a role in this motile system. We would like to propose that UM has an adhesive function not only on early embryos but also on adult intestinal cells. Uvomorulin might serve as an adhesive factor that provides a counter-force against the tension of the contractile ring, thereby helping to preserve the integrity of the epithelial sheet.

The results presented in this report encourage a more precise electron microscopic study of UM on preimplantation embryos. As previously mentioned, it has been reported by ultrastructural analysis that UM is uniformly distributed on the surface of EC cells (8), but these studies have so far not been extended to early embryos. It is the process of morula compaction, a prerequisite for blastocyst formation, with which anti-UM antibodies interfere. Though in early morulae only gap junctions can be detected, other intercellular junctions appear in the outer cell sheet of late morulae and blastocysts (2). We are now investigating whether the distribution of UM changes during the morula-blastocyst transition and whether UM localization is related to the appearance of cell junctions.

We thank I. Zimmermann for preparing ultrathin sections and R. Groemke-Lutz and S. Haase for help with the micrographs. The help of Dr. T. Doetschman in critically reading and thereby improving the manuscript is gratefully acknowledged, as is the typing of the manuscript by R. Brodbeck.

Received for publication 15 June 1984, and in revised form 1 October 1984.

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