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A Collagen Substrate Enhances the Sealing Capacity of Tight Junctions of A6 Cell Monolayers

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Abstract. A6 cells, a kidney derived epithelial cell line, when cultured either on a collagen-coated substrate or on polycarbonate substrate without collagen form confluent monolayers that are similar in cell density and overall morphology. However, the transepithelial electrical resistance (TER) of monolayers grown on the collagen-coated substrate is ninefold higher than that of monolayers grown without collagen. A comparative freeze-fracture study showed that this large difference in TER is not related to the length or number of tight junction strands but to differences in the specific conductance of individual strands. This conductance was obtained considering the TER, the linear junctional density and the mean number of tight junction strands. We estimated the specific linear conductance of the tight junction strands to be 2.56×10^{-7} S/cm for cells grown on collagen and 30.3×10^{-7} S/cm for the cells grown without collagen. We also examined changes in distribution and phosphorylation states of the zonula occludens associated protein, ZO-1, during monolayer formation. Immunocytochemistry reveals that the distribution of ZO-1 follows a similar time course and pattern independent of the presence or absence of collagen. While the amount of ZO-1 expression is identical in cells grown on both substrates, this protein is phosphorylated to a greater extent during the initial stages of confluence in cells cultured on collagen. We suggest that the phosphorylation levels of ZO-1 in A6 cells at the early stages of monolayer formation may determine the final molecular structure and specific conductance of the tight junctions strands.

Key words: Tight junction — Intercellular junctions — Transepithelial permeability — Transepithelial electrical resistance (TER) — A6 epithelial cell cultures — Collagen.

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Introduction

The tight junction is a structure in the plasma membrane that provides a selectively permeable occlusion along the paracellular pathway across polarized epithelial cell monolayers. This selectively permeable occlusion is necessary to maintain vectorial secretion, absorption and transport. The tight junction can be recognized in an epithelium either by its physiological properties or its morphology in electron micrographs. In freeze fracture replicas, the junctions appear as transmembrane strands which are linked together in the plane of the intercellular space (Staehelin, 1973). The sealing capacity of the tight junction can be evaluated by measuring the transepithelial electrical resistance (TER) (Frömter & Diamond, 1972; Diamond, 1977).

The sealing capacity of tight junctions in natural epithelia and cultured monolayers depends not only on the length and number of tight junction strands (Claude & Goodenough, 1973; Claude, 1978), but also on several other factors. The existence of channels (Claude, 1978; González-Mariscal, Chávez de Ramirez & Cereijido, 1984), the compartmentalization afforded by the frequent anastomoses between junctional strands (Cereijido, González-Mariscal & Contreras, 1989), the biochemical state of junctional components (e.g., phosphorylation) (Stevenson et al., 1988, 1989), the control provided by the relationship between the tight junction and the cytoskeleton (Montesano et al., 1975, 1976; Saxon et al., 1978; Bentzel et al., 1980; Meza et al., 1980, 1982; Madara, Barenberg & Carlson, 1986; Hecht et al., 1988)

and other factors (Cereijido, 1991) can influence the TER. Tight junction permeability, like the vectorial transport mechanisms of the cell, is affected by cell-substrate interactions (Cott, 1989). Whether this influence is secondary to changes in cell shape that alters the length of tight junctions or is due to an alteration in the intrinsic properties of the tight junction itself remains to be determined in each case (Schneeberger & Lynch, 1992).

We found that A6 cells, a kidney-derived epithelial cell line, cultured in either the presence or absence of collagen in the substrate form confluent monolayers similar in cell density and overall morphology within 5–7 days. However, there is a 9-fold difference in TER depending on whether or not the culture substrate contains collagen. We used these two culture conditions to investigate the relationship between epithelial permeability and tight junction structure. We found that this large difference in TER is based on differences in specific conductance of the tight junction strands. We also investigated the changes in phosphorylation of the zonula occludens protein, ZO-1 (Stevenson et al., 1986; Anderson et al., 1988), that accompany the formation of the monolayers. We suggest that the phosphorylation levels of ZO-1 in A6 cells at the early stages of monolayer formation may determine the final molecular structure and specific conductance of the tight junctions strands.

Materials and Methods

Cell Culture and Electrical Measurements

A6 cells (CCL 102) obtained from American Type Culture Collection (Rockville, MD) were grown at 26°C in CL2-Amphibian medium (NIH-Media Section, Bethesda, MD), 10% fetal bovine serum and 2 mM glutamine Pen-Strept (Irvine Scientific, Santa Ana, CA) in an air-1% CO_2 atmosphere. Cells at confluence were harvested with 0.25% trypsin solution (GIBCO-BRL, Grand Island, NY). The cell suspensions were plated at the same density, enough to reach confluence in several hours, on 6-well plates with Transwell cell culture inserts (4.7 cm^2 growth area and 0.4 μm pore size; Costar, Cambridge, MA). Two types of substrates were used: polycarbonate filters (Transwell, Costar) and collagen-treated filters that contained a mixture of collagen types I and III (Transwell COL, Costar).

The TER across the A6 cell monolayers grown on polycarbonate filters and on collagen-treated filters was measured using an epithelial voltohmmeter (World Precision Instruments, New Haven, CT) operated at a constant current of 20 μA. The electrical resistance of the filter and of the media was subtracted from the measured values and expressed for the area of the filter (4.7 cm^2).

To determine the cell density and the linear junctional density (the linear density of cell contacts per square centimeter of cell monolayer) (González-Mariscal, 1991) we labeled the monolayers with rhodamine-phalloidin (Molecular Probes, Eugene, OR) diluted in 0.5% PBS-Triton X-100 for 1 hr at room temperature. Clear cellular profiles and cell borders could be visualized. Mophometric analysis of the junctional region was done by tracing the images corresponding to the cell borders of the confluent monolayers using the NIH Image 1.49 software.

Freeze-Fracture and Morphometric Analysis

For freeze fracture, the cell monolayers attached to the filter inserts were fixed by immersion in 2% glutaraldehyde in phosphate buffer saline (PBS) for 2 hr, washed thoroughly in PBS, scraped from the surface of the filter, impregnated in 30% glycerol, mounted and frozen by immersion in liquid Freon 22. Specimens were freeze-fractured at −110°C in a Balzers 301 freeze-fracture apparatus, shadowed with platinum-carbon and viewed in a JEOL 100 CX II electron microscope.

Morphometric analysis of tight junction strands was performed according to a previously described procedure (Bentzel et al., 1980; Lagarde et al., 1981; González-Mariscal et al., 1984; Jaeger, Dodane & Kachar, 1994). The micrographs of the freeze-fracture replicas were printed at 70,000 × magnification and were overlapped with a transparent sheet containing a test pattern. This test pattern consisted of one line which was placed parallel to the main axis of the junction and a series of perpendicular lines drawn at 5 mm intervals. The number of tight junction strands per cross section was defined by the number of strands intersecting each perpendicular line crossing the junction. Tight junction depth was the distance between the most apical and the most basal strands intersecting each perpendicular line. Zero depth was assigned to a single strand junction. Results are expressed as the mean ± (SEM). Differences between the data were assessed by the Student's unpaired *t* test.

ZO-1 Immunolocalization

Cell monolayers grown on filters were fixed with 1% paraformaldehyde in PBS, pH 7.4, for 10 min at room temperature. After fixation, the cells were permeabilized with 0.5% Triton X-100 in PBS, followed by an incubation with 1% bovine serum albumin (BSA) in PBS. The affinity-purified rabbit antibody against ZO-1 (Zymed Lab, San Francisco, CA, 1 mg/ml in PBS, with 0.05% NaN_3, pH 7.4) was applied and then visualized with fluorescein-conjugated donkey anti-rabbit IgG (Amershan, Arlington Heights, IL). Antibody incubations were done for 45 min at room temperature. The dilutions were 1:300 for the primary antibody and 1:100 for the secondary. After each incubation, the cell monolayers were washed in PBS containing 0.5% Tween-20. After labeling, filters were removed from the filter inserts and sandwiched between a glass slide and a coverslip in mounting media. The observations and photographic recording were performed under a Zeiss Axiophot fluorescence microscope. The objectives used were a 63X Plan Apochromatic, 1.4 NA and a 40X Plan Neofluor, 1.3 NA.

ZO-1 Immunoprecipitation

Confluent monolayers at 1, 3, 5 and 7 days in culture were rinsed twice with phosphate and serum-free CL2-amphibian medium and left overnight in the same medium to starve cells of phosphate. Labeling was done for 12 hr at room temperature in the same phosphate and serum-free CL2-amphibian medium with the addition of 250 μCi of [^{32}P] orthophosphate per ml (carrier-free, 285 Ci/mg). The labeling medium was discarded, and the filters were rinsed twice with cold PBS before solubilizing total cell protein in lysing buffer (50 mM TRIS, pH 7.2 to 7.8; 1% NP-40 or 1% Triton X-100; 2 mM EDTA; 100 mM NaCl; 1 mM Vanadate; 1 mM PMSF; 0.1% Aprotinin). Cells were then scraped from the filters, vortexed and centrifuged. ZO-1 was immunoprecipitated from the supernatant using an anti-ZO1 polyclonal antibody (Zymed) with the aid of protein G-Sephadex (Pharmacia LKB Biotech., Piscataway, NJ). Samples were boiled in loading buffer, electrophoresed on 12% (w/v) SDS-polyacrylamide gels, transferred to PVDF membranes and labeled with the polyclonal anti-ZO-1 (Zymed) diluted

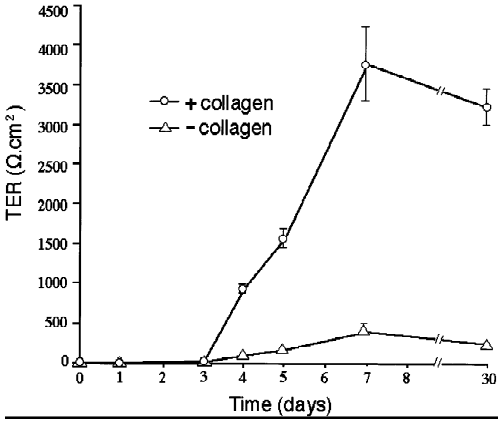

Fig. 1. Time course of TER of A6 cells plated on collagen coated substrate (—○—) and on plain polycarbonate substrate (—△—). On day 4, the TER of cells plated on collagen-treated substrate averaged 925.2 ± 50.1 $\Omega.cm^2$, while the cells plated on plain polycarbonate substrate averaged 91.7 ± 3.2 $\Omega.cm^2$. The TER continued to increase up to day 7 when they averaged 3,763 ± 464 $\Omega.cm^2$ and 403 ± 41 $\Omega.cm^2$ respectively. Each data point represents the average value for a minimum of six monolayers.

1:2000 in TBS-BSA, followed by ^{125}I-protein A (2 μCi/ml). The ZO-1 bands were excised from the membranes and then counted for both ^{32}P and ^{125}I in a liquid scintillator, using counting-chamber limits such that no significant overlap between isotopic emissions occurred. The ratio of these radioactivity counts (^{32}P/^{125}I) represents the specific activity of phosphate on ZO-1 (Fig. 4). Each data point in Fig. 4 was obtained by combining the ZO-1 immunoprecipitated from A6 cells from three culture filters and run together in a single lane in the SDS-plyacrilamide gel.

Results

Cell Culture and Electrical Measurements

The time courses of TER of monolayers grown on plain polycarbonate substrate and collagen-treated substrate are shown in Fig. 1. Development of the TER was similar for cells grown on both substrates until the third day after seeding. When measured on the fourth day after seeding, the TER of monolayers grown on collagen-treated filters averaged 925.2 ± 50.1 $\Omega.cm^2$, while the TER of monolayers grown on polycarbonate filters averaged 91.7 ± 3.2 $\Omega.cm^2$. At day 7, the TER values measured were 3,763 ± 464 $\Omega.cm^2$ for cells grown on collagen-treated filters and 403 ± 41 $\Omega.cm^2$ for cells grown on polycarbonate filters.

Actin labeled by rhodamine phalloidin outlined the A6 cells profiles (*not shown*). These profiles yielded data for determining the measurements of both the cell density (number of cells per square centimeter) and the linear junctional density (linear amount of cellular contact per square centimeter). The A6 cells grown on both substrates formed monolayers with similar cell densities and linear junctional densities (Table 1).

Tight Junction Morphology

The freeze-fracture replicas of 7-days-old A6 cells grown on the two types of substrates showed the usual configuration of tight junctions. They consisted of a strand meshwork that is predominantly oriented parallel to the luminal surface (Fig. 2). The strands appear as intramembrane fibrils on the protoplasmic (P) fracture face and the characteristic complementary grooves on the exoplasmic (E) fracture face (Fig. 2). The number of strands and the tight junction depth which is the distance between the most apical and most basal strands of the tight junction strand network are shown in Table 1. The tight junction strand network of cells grown on collagen showed a statistically significant smaller mean number of strands (2.53 ± 0.05) than that of cells grown in the absence of collagen (3.09 ± 0.05). In addition, the mean value of tight junction depth (Table 1; Fig. 2) for cells grown on collagen was also significantly smaller (0.115 ± 0.004 μm) that for A6 cells grown on the absence of collagen (0.201 ± 0.006 μm).

To better understand the differences between the two monolayers, we expressed our electrical results as conductance which is the reciprocal of the TER. Transepithelial conductance (expressed as S/cm^2) is a reliable parameter of the paracellular conductance, reflecting the degree of junctional sealing. The values of monolayer conductance obtained were 2.66×10^{-4} for the monolayers plated on collagen-coated substrate and 24.8×10^{-4} for the monolayers plated on non-coated polycarbonate substrate (Table 2). By dividing these values of monolayer conductance by the values of linear junctional density (Table 1) we estimated the linear junctional conductance. The linear junctional conductance calculated for the monolayers formed on collagen-coated substrate was 1.02×10^{-7} S/cm and for the monolayers formed on non-coated polycarbonate substrate was 10.0×10^{-7} S/cm (Table 2). Finally, when we correct the linear junctional conductance for the mean number of tight strands, we obtain the specific linear conductance for individual strands. The calculated specific linear junctional conductance of individual strands was 2.56×10^{-7} S/cm for the monolayers grown on collagen-coated substrate and 30.3×10^{-7} S/cm for the monolayers formed on uncoated polycarbonate substrate.

ZO-1 Immunolocalization

The changes of ZO-1 distribution during the formation of the monolayer are shown in Fig. 3. Cells grown on collagen and cells grown in the absence of collagen show a similar pattern of progressive accumulation of ZO-1

Table 1. Structural aspects of tight junctions of A6 cells grown in the presence and in the absence of collagen on the substrate

Parameters	Collagen (+)	Collagen (−)
Cell density (10^5 cells/cm^2)	12.33 ± 0.13 (17)	12.08 ± 0.37 (8)
Linear junctional density (cm/cm^{-2})	2606 ± 17 (17)	2470 ± 31 (8)
Mean number of tight junctions strands	2.53 ± 0.05 (49)	3.09 ± 0.05* (47)
Mean depth of tight junctions (μm)	0.115 ± 0.004 (49)	0.201 ± 0.006* (47)

(n) = number of micrographs
* Significantly different from collagen (+) A6 cells ($P < 0.001$)

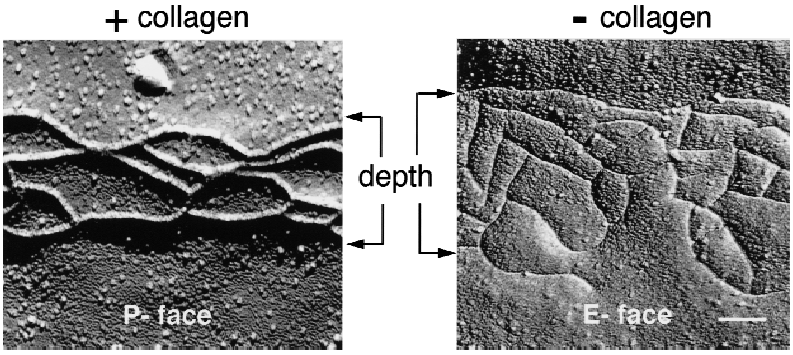

Fig. 2. Freeze-fracture replicas of tight junctions from A6 cells grown on collagen-coated-substrate and on plain polycarbonate substrate. The tight junction appears in protoplasmic (P) and exoplasmic (E) fracture faces as a network of long parallel strands, and short interconnecting strands. (Bars = 0.10 μm).

Table 2. Specific conductance of tight junctions of A6 cells grown in the presence and in the absence of collagen on the substrate

Parameters	Collagen (+)	Collagen (−)
TER of the monolayers (Ω cm^2)	3,763 ± 464	403 ± 41
Conductance of the monolayers (S/cm)	2.66×10^{-4}	24.8×10^{-4}
Linear junctional conductance (S/cm)	1.02×10^{-7}	10.0×10^{-7}
Specific linear conductance for individual tight junction strands (S/cm)	2.56×10^{-7}	30.3×10^{-7}

along the cell borders. After 1 day in culture ZO-1 appears as a punctated pattern along regions of cell to cell contacts. The amount of label progressively increases and after 3 days starts to form a linear pattern that follows the outline of the contacting cell borders. Finally at day 7 the ZO-1 is completely organized in the characteristic honeycomb pattern in both culture conditions.

ZO-1 Immunoprecipitation

We have examined the level of phosphorylation of ZO-1 in A6 cells grown with or without collagen at 1, 3, 5 and 7 days after plating. The $^{32}P/^{125}I$ ratios of these differentially cultured A6 cells were determined (Fig. 4). The ratio of radioactivity counts ($^{32}P/^{125}I$) represents the specific activity of phosphate of ZO-1. At the first stages of the cell monolayer establishment, ZO-1 of A6 cells grown on collagen contains up to three times more phosphate than do A6 cells grown in the absence of collagen. This difference in the ZO-1 phosphate content decreased gradually over time and had essentially disappeared at the final stage of the cell monolayer establishment, when the monolayers had also reached TER steady states.

Discussion

We report here that the A6 epithelial cells, when cultured on a collagen-coated substrate form monolayers with a transepithelial electrical resistance (TER) nine times higher than such cells plated on an uncoated substrate. In spite of this large difference in TER, the overall morphology of A6 cell monolayers grown on either collagen or plain substrate is similar as is the junctional density. Surprisingly, the tight junction of cells grown on collagen-coated substrate presented a smaller mean number of strands than did junctions of cells grown on the uncoated

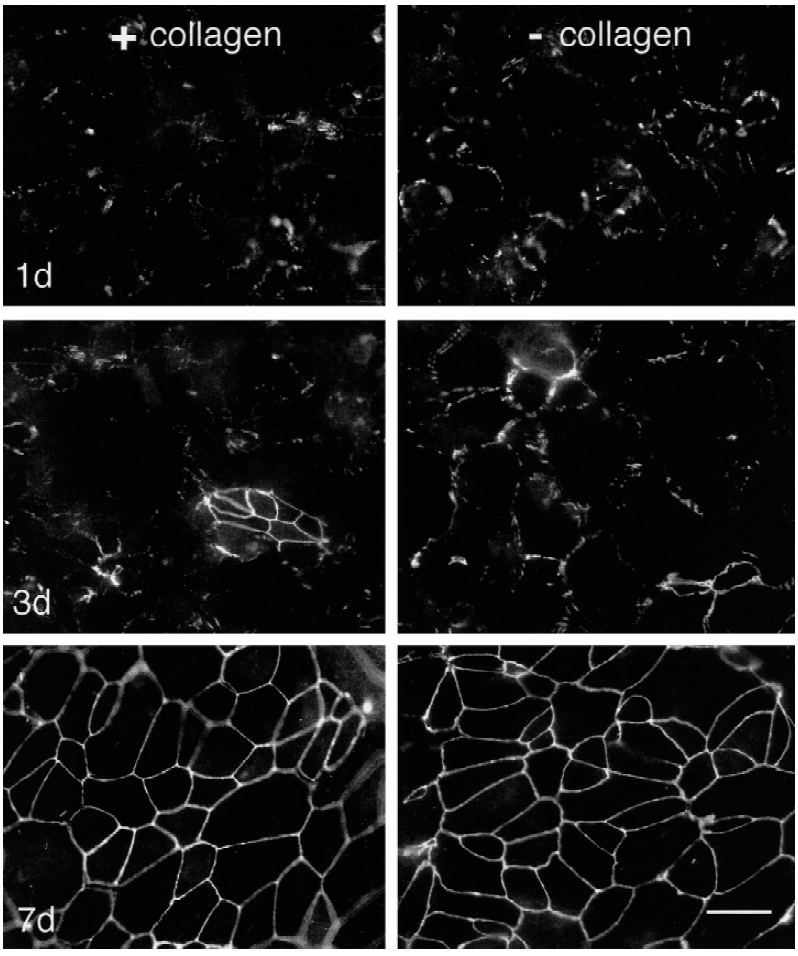

Fig. 3. Immunofluorescence localization of ZO-1 in A6 cells grown on collagen-coated-substrate (left) and on uncoated polycarbonate substrate (right). After 1 day in culture (1d) ZO-1 appears as a punctated pattern along regions of cell-to-cell contacts. The amount of label progressively increases and after 3 days (3d) starts to form a continuous linear pattern that follows the outline of the contacting cell borders. Finally at day 7 the ZO-1 is completely organized in the characteristic honeycomb pattern in both culture conditions (7d).

substrate. On the other hand we did not detect any differences in the morphology of the individual strands of cells grown in either conditions.

The wide range of values of transepithelial resistence reported in a variety of epithelia is largely due to differences in the junctional resistance. Typically, the paracellular pathway is much more conductive (more than an order of magnitude) than the transcellular route (Gonzalez-Mariscal, 1991). There are very rare exceptions to this rule in which the electrical resistance of the cell membranes are unusually low (Augustus et al., 1977) but in most cases the TER reflects the paracellular value (Reuss, 1991). For very high resistance epithelia (>3,000 $\Omega.cm^2$), changes in the transcellular ionic permeability could account for a fraction of the changes in the measured TER values. However, in our experiments, the TER in the absence of collagen drops to very low values (~400 $\Omega.cm^2$) which cannot be explained by a change in transcellular ionic permeability and is rather like the typical changes associated with the tight juntion-mediated paracellular permeability (Gonzalez-Mariscal, 1991). The possibility that the very low TER seen in the absence of collagen, is due to an unusual transcellular permeability can be excluded based on recent preliminary experiments by F. Lacaz-Vieira et al. (*personal communication*) in which the paracellular conductance is partially blocked by the selective deposition of $BaSO_4$ at the tight junctions (Castro, Sesso & Lacaz-Viera, 1993). The simple $BaSO_4$ deposition at the tight junctions in these monolayers decreased the transepithelial conductance from $14.3 \pm 2.3 \times 10^{-4}$ S/cm^2 to $6.7 \pm 1.1 \times 10^{-4}$ S/cm^2 (F. Lacaz-Vieira et al., *personal communication*). This means that at least 50% of the conductance is due to paracellular route and rules out the possibility that the transcellular conductance dominates the overall conductance in these cell monolayers.

To better analyze the differences between the tight junctions we converted all our TER values into conductance values. The calculated conductance for the strands is significantly smaller in monolayers grown on collagen. The estimated specific linear conductance of the strands were 2.56×10^{-7} S/cm for cells grown on collagen and 30.3×10^{-7} S/cm for cells grown in the absence of collagen. The structure of the tight junction strands, analyzed in the freeze-fracture replicas, did not show any difference between the monolayers grown in both con-

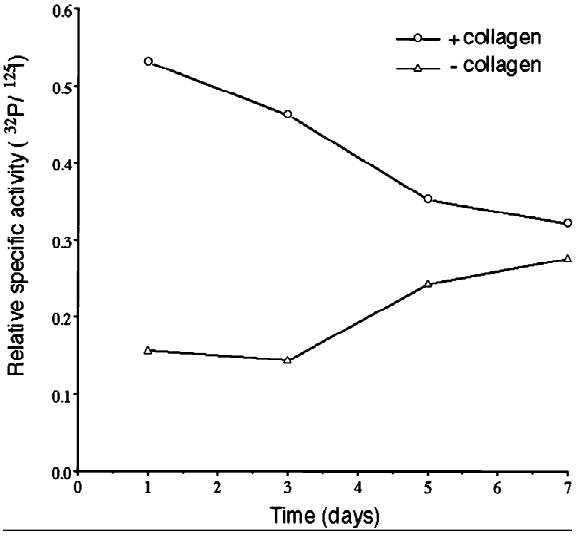

Fig. 4. Time course of the specific activity of phosphate on ZO-1 for A6 cells grown on collagen-coated substrate (—○—) and on non-coated polycarbonate substrate (—△—). At the early stages of confluence ZO-1 is more phosphorylated in the cells seeded on the collagen-treated substrate. Later on the phosphorylation levels become comparable.

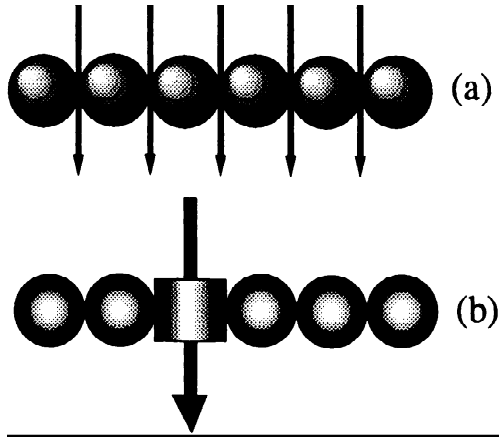

Fig. 5. Schematic representation of two possible ways paracellular current can flow through the tight junction strands: (*a*) current would pass through low conductance channels through or around each structural subunit of the tight junction; (*b*) current would pass through rare high conductance pores intercalated in the structure of the strand.

ditions. Thus the basis for the difference in conductance of the strands is at a molecular level, not resolvable by the freeze-fracture technique.

The two most likely ways that paracellular current would pass through tight junction strands are shown in Fig. 5. If the tight junction strand is formed by a linear array of subunits, each of them could contribute equally to the overall linear conductance of the strands (Fig. 5a). Previous reports have shown that the strands are made of 10 nm subunits (Staehelin, 1973; Lane, Reese & Kachar, 1992). Using this value, we calculated a hypothetical value of conductance associated with each subunit assuming that each pathway for the current flow is permanently open. The values that we obtained were 0.26 pS for the cells seeded on the collagen-coated substrate and 3.0 pS for the cells plated on the plain polycarbonate substrate. These values are at a lower range of the measured conductance (2–200 pS) for most membrane ion channels (Hille, 1992). For each of these putative channels, associated with these subunits, to have a conductance comparable with the membrane ion channels they would have to have a gating mechanism and be closed for some of the time. A second possibility would be the existence of rare high conductance junctional pores (Madara, 1991) intercalated in the structure of the strand (Fig. 5*b*). It is easier to explain our results of a tenfold difference in the specific conductance of the strands if the second hypothesis is correct. In that case, the cells grown on the collagen-coated substrate would have fewer putative channels along the strands. For the moment, these hypothetical considerations are only an attempt to combine the structural analysis with the result of junctional permeability. To fill the gap in our knowledge of the regulation of the tight junction conductance will require the identification of such paracellular channels and the experimental determination of their associated single-channel conductances.

As an initial step to investigate the molecular basis for the permeability differences of the tight junctions in these monolayers, we analyzed the changes in the expression of the zonula occludens associated protein ZO-1 during monolayer establishment. ZO-1 is a phosphoprotein (Stevenson et al., 1986) involved in multiple protein-protein interactions and has been implicated in the regulation of the tight junction permeability (Anderson, Balda & Fanning, 1993). We followed the changes in distribution and phosphorylation levels of the protein ZO-1 during junction formation in the A6 cells. In both culture conditions ZO-1 appears in the cytoplasm at the regions of contact, as the cells become confluent. While the total amount of ZO-1 is the same, this protein is phosphorylated to a greater extent in cells grown on the collagen substrate during the initial stages of monolayer establishment. The difference in phosphorylation levels was observed only at the initial stages of formation of the monolayer, while the large difference in TER appears several days later. The confluence of the cells is complete 7 days after seeding, when ZO-1 assumed the continuous honeycomb pattern.

There are data indicating that pharmacological manipulation of kinases systems affects the permeability properties of the tight junction (Duffey, Hainau & Bentzel, 1981; Ojakian, 1981; Mazaraiegos, Tice & Hand, 1984). It is not known how the phosphorylation of the ZO-1 protein could influence the structure and specific conductance of the strands. ZO-1 interacts directly with

occludin, a putative integral membrane protein of the tight junction (Furuse et al., 1994) and it is possible that from this interaction the subunits across the strands pack to form the ion selective pores in the extracellular space (Gumbiner, 1993). Phosphorylation levels of ZO-1 could affect this interaction, leading to different packing of the tight junction subunits of modifying the amount or the permeability of the putative tight junction paracellular channels (Madara, 1991). On the other hand, Balda et al. (1993) have shown in MDCK cells that the phosphorylation state of ZO-1 does not change with the rapid changes in TER in the Ca^{2+} switch experiment. They conclude that the rapid recovery of the permeability barrier in their experiments is based on the simple reassembly of pre-existing tight junction complexes. Our results also show that the phosphorylation level of ZO-1 does not always correlate directly with tight junction permeability since we observed that the low conductance A6 monolayers (grown on the collagen-coated substrate) and the high conductance A6 monolayers (grown on the uncoated substrate) show the same final ZO-1 phosphorylation levels. However, in addition, our results suggest that the phosphorylation levels of ZO-1 in A6 cells at the initial stages of cell monolayer establishment may indirectly influence the determination of the final molecular structure and permeability of tight junctions.

It is known that cell-cell and cell-extracellular matrix interactions are critical for the development of epithelial organization (Cereijido, 1991). Tight junctions are known to be structurally and functionally associated with cytoskeletal elements (Meza et al., 1980, Hecht et al., 1988; Madara et al., 1986). As ZO-1 appears in the cytoplasma near the points of initial cell-cell contacts, it may be involved in mediating cytoskeletal assembly signals (Anderson et al., 1993). Our results also show that the tight junctions between cells cultured on collagen substrate form a more compact network of strands (narrower tight junction depth). Thus, it is possible that rearrangements of the strands, with the compaction of the tight junction network, depends on a concerted rearrangement of cytoskeletal elements. In fact, alterations in the cytoskeletal components at the apical border of epithelial cells have been associated with increases in the TER values (Bentzel et al., 1980). A similar compaction of tight junction strands as observed here was reported to be associated with an increase of TER in cultured cells (Jaeger et al., 1994). The establishment of the precise molecular mechanism that generates differences in TER will depend on further understanding the effect of the cell substrate on the epithelial cellular organization.

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