EFFECT OF CELL-SUBSTRATUM INTERACTION ON HEMICYST FORMATION BY MDCK CELLS

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SUMMARY

On impermeable substrata MDCK cells, a cell line derived from normal dog kidney, forms a confluent monolayer that is studded with numerous hemicysts. Previous studies with this cell line suggest that these hemicysts develop as a result of active fluid accumulation between cell sheet and substratum. However, the formation of hemicysts as a multifocal phenomenon is still unexplained. The results presented here show that the hemicysts are not only expressions of active transport of solutes and water, but also of cell-substratum interaction. The increase in number and size of the hemicyst produced by dbcAMP may be explained by a decrease in the adhesive strength to substrata produced by this compound. Moreover, when the strength of the cell-substratum adhesion was increased the number of hemicysts was reduced or abolished. On the contrary, when this strength was reduced, larger hemicysts occurred, covering practically all the area available for growth. Results from cinematographic time lapse studies, showing that 90% of the area of the monolayer is able to produce hemicysts, also suggest that hemicyst formation as a multifocal phenomenon is more an expression of local variations in cell-substratum interaction than of regional changes in transepithelial active transport.

Key words: MDCK cells; cell-substratum interaction; hemicyst formation; transepithelial active transport; dbcAMP.

INTRODUCTION

The MDCK cell line is an epithelial cell line derived from normal dog kidney (1). On solid and impermeable support these cells grow as a confluent monolayer that is studded with numerous "hemicysts" also termed "domes" or "blisters" (2). Since a variety of epithelial cell cultures have also been found to produce multicellular hemicysts (3-10) the formation of hemicysts seems to be a common phenomenon among epithelial cells in culture. Previous studies indicated that hemicyst formation is associated with active transport of solutes and water, resulting in a local accumulation of fluid between the cell sheet and the substratum (11).

MDCK cells grown on a permeable support provide a cell culture model that exhibits several characteristics of in vivo epithelial membranes (12-14). The simplicity of the model and the possibility to reverse from isolated cells to a complete monolayer makes it an ideal tool for studying several problems of the physiology and cell biology of epithelial membranes.

Since the presence of hemicysts on an impermeable support is an expression of active transport of solutes and water, their scattered appearance in a monolayer introduces some uncertainty of the homogeneity of the transport properties of this monolayer. Some factors that may determine why hemicysts appear only in localized sites of the monolayers are: interruption of zonula occludens in areas without hemicysts, local variations in the active transepithelial transport, differences in adhesion between cells and substratum, or differences in cell density. Previous studies have shown
that the zonula occludens encircling MDCK cells can provide an effective permeability barrier with characteristics similar to those of a leaky epithelium (12,13,15). We also have shown that these zonula occludens have the same continuity and properties in hemicyst and nonhemicyst areas (15). In spite of 300% stimulation in the number of hemicysts produced by dibutyryl cyclic AMP (dbcAMP), the specific binding of $[^3H]$ouabain is the same in control and dbcAMP-treated cultures (14). The correlation found between $[^3H]$ouabain binding and transepithelial active transport (16), together with the inhibition of the active transport of solutes and water produced by cyclic AMP (cAMP) in leaky epithelia (17-20), suggest that factors other than the variations in the active transepithelial transport would be involved in the localized hemicyst formation. In this paper we describe the formation and abolition of hemicysts by altering the interaction between MDCK cells and the solid substratum.

**MATERIALS AND METHODS**

**Maintenance of cell line.** MDCK cells were maintained by serial passage in stoppered 32-oz prescription bottles at 36°C. The cultures were fed with Eagle’s minimal essential medium (Microbiological Associates, Bethesda, MD). The medium was supplemented with 15% bovine serum, L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (50 µg/ml). When cell growth reached saturation density, subcultures were prepared using 0.02% EDTA:0.05% trypsin (GIBCO, Grand Island, NY).

**Preparation of hemicysts and monolayer.** Cultures of hemicysts and monolayer were prepared in large Leighton tubes containing a sterile standard glass microscope slide. Each tube was inoculated with 10 ml of cell suspension of approximately $10^5$ cells/ml to reach a cell density of $5 \times 10^5$ cells/cm². The cultures were fed every 2 or 3 days with 10 ml of medium during 10 days prior to the electrical measurements.

Monolayers on a permeable support were prepared using a polycarbonate filter membrane with 5-µm pore size and 25-mm diameter (Nucleopore Corp., Pleasanton, CA), both faces of which had been covered with a very thin film of 1% collagen dispersion (Ethicon, Somerville, NJ) and applied to a glass slide. The methods for collagen aggregation into native bundles and sterilization of the collagen-coated membrane are described elsewhere (15).

**Solutions.** Hanks’ solution was used for dilution potential determination. The standard Hanks’ solution has the following composition (mM): 136.8 NaCl, 5.63 KCl, 1.26 CaCl₂, 0.49 MgCl₂, 0.45 MgSO₄, 4.16 NaHCO₃, 0.33 Na₂HPO₄, 0.44 K₂HPO₄, 5.5 glucose, pH 7.42. Isosmotic dilutions were prepared by mixing equal volumes of Hanks’ complete solution and Hanks’ solution in which all of the NaCl was substituted isosmotically by sucrose. Bi-ionic potentials were measured by replacing 136.8 mM NaCl with 136.8 KCl, LiCl, RbCl, CsCl, or choline Cl. All these solutions were supplemented with 15% bovine serum of known electrolyte composition. The final ionic concentration was calculated taking into account the serum contribution. All solutions were prepared from reagent grade chemicals. The electrolyte composition was checked using simultaneous multiple analyzer model SMA-6 (Technicon Instruments Corp., Tarryton, NY).

**Electrical measurements.** The collagen-coated filter with the monolayer was removed from the culture tube and mounted as a flat sheet between two Lucite half chambers with a window area of 3.14 cm². Transepithelial potential differences were measured as described before (15) using an Orion model 701 pH meter. The current was measured with a CSC microammeter model 320 G (Triplett Corp., Bluffton, OH) and was conducted by AG/AgCl electrodes on opposite sides of the membrane and at the rear of the chamber. All experiments were performed at 25°C. The inside solution was Hanks’ complete solution. To obtain the dilution or bi-ionic potentials, changes in the composition were made in the outside solution even though the same numerical values were obtained by changes in the inside solution. For conductance measurements, an outside solution identical in composition to the inside solution was used. The potential difference values ($\Delta \omega$) were obtained every 30 s until a steady or quasi-steady state was achieved.

**Changes in cell-substratum interaction.** To study the role of the adhesive strength in different cellular functions, two methods have been commonly used: by modification of substratum adhesiveness (21) or by isolation of cell attachment mutant (22). We have used the first method in our study.

To increase the adhesive strength, MDCK cells were grown on glass cover slips, half of which had been treated with glutaraldehyde-activated 3-aminoopropyltriethoxysilane and the other half