DEMONSTRATION OF PROTEIN ASYMMETRIES IN THE PLASMA MEMBRANE OF CULTURED RENAL (MDCK) EPITHELIAL CELLS BY LACTOPEROXIDASE-MEDIATED IODINATION

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1. Introduction

Epithelial cells, such as the enterocyte and the renal proximal tubule cell, transport solutes (Na^+, Cl^-, sugars, amino acids) in a vectorial fashion across the epithelial cell layer [1]. This polarity of function is reflected in polarity of cell structure (e.g., microvilli, basal infoldings and lateral cellular spaces) and in a plasma membrane that consists both in protein and lipid composition, of two distinct regions [2,3] (the apical or brush-border membrane and the basal-lateral membrane) separated by a junctional complex [3].

There are two important questions still unanswered regarding this membrane specialisation:

(1) Since most of the information about protein/enzyme locations has depended upon subcellular fractionation requiring centrifugation [3,4] or upon free-flow electrophoresis [3,5] yielding only partially purified preparations, the extent to which the protein composition of each membrane region differs is still unanswered;

(2) The factors controlling the formation and maintenance of such specialised membrane domains are unknown.

Here we report observations concerning the in situ determination of the protein composition of the two membranes of cultured renal (MDCK) epithelial cells. Cells of this continuous cell-line (MDCK) grown to form a continuous monolayer of cells upon permeable supports have been shown to maintain morphological and functional features similar to an in vivo renal epithelium [6–9]. In addition, the preferential localisation of the Na-K ATPase to the basal-lateral membranes has been demonstrated [10]. The method employed utilises the structural simplicity of the cultured epithelial system; enzymic radioiodination being used to label externally-exposed brush-border or basal-lateral membrane proteins selectively.

2. Materials and methods

2.1. *Cell culture*

MDCK renal epithelial cells at 60 serial passages (Flow Lab.) were grown as in [6]. Cell monolayers were prepared upon 2.5 cm diam. Millipore filters (0.22 μm pore diam.) by seeding at high density [7] followed by growth to confluency. Monolayers were used after 3–4 days growth. Measurements of electrical resistance and active transport potential [11] were made from monolayers from the same batch used for iodination.

2.2. *Iodination*

Cell monolayers were first rinsed and incubated in Eagle's salt solution for 1 h at 37°C with agitation, then clipped into polythene mini-Marbrook Chambers (Hendley Co.) allowing access to either apical or basal surfaces. (Monolayer integrity as judged by electrical resistance was not affected.) The iodination of externally-exposed protein was initiated by addition of 0.25 cm^3 of iodination mixture to the appropriate surface (250 μCi $Na^{125}I$, 2 nmol H_2O_2, 1.25 μg lacto-

peroxidase in Hank's solution); iodination was for 30 min at 37°C. Preincubation of the basal surfaces with 1 cm^3 5 $\mu g/cm^3$ lactoperoxidase in Hank's solution was necessary to ensure adequate iodination of the basal-lateral surfaces.

Subsequent to iodination, monolayers were washed in ice-cold Hank's solution with 10 μM sodium sulphite for 30 min to remove unbound ^{125}I. Incorporation of ^{125}I into protein was > 5% and was 99% dependent upon H_2O_2. Non-specific (lactoperoxidase-independent) iodination was < 2% of total incorporation.

2.3. *SDS–polyacrylamide gel electrophoresis*

Samples were solubilised and electrophoresed in 16% polyacrylamide slab gels using the discontinuous

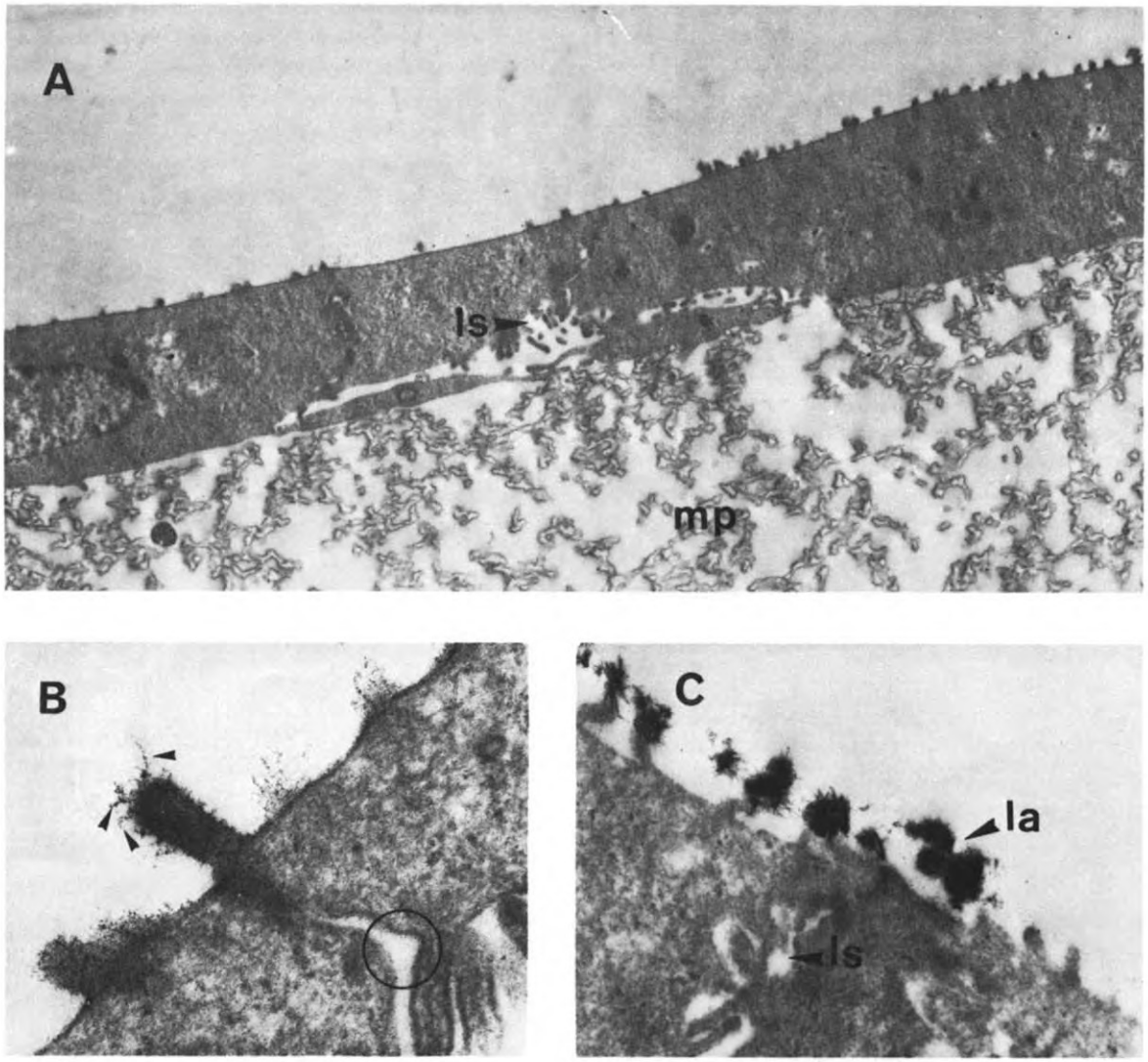

Fig.1. (A) Low power view (× 7830) of a confluent monolayer of MDCK cells grown upon a millipore filter (MP). Note the sparse brush-border (apical surface) and the lateral space (LS) between cells (basal/millipore surface). (B) High power (× 79 170) view of an apical junctional region between two cells. Note that apical microvilli possess a glycocalylx (arrowed) whereas interdigitations within the lateral space are bare (circle). (C) Penetration of the tight junction by the tracer molecule La^{3+}, perfused from the apical or brush-border surface is not observed. La^{3+} is not detectable in the lateral spaces (LS) (× 39 150).

buffer system [12]. Fixing and staining was according to [13]. Molecular weight markers were *Escherichia coli* RNA polymerase, bovine serum albumin, and soybean trypsin inhibitor. Autofluorography was performed on dried gels [14] using Kodak X-Omat H film.

2.4. *Electron microscopy*

Cell monolayers were conventionally fixed and embedded. Thin sections were stained with uranyl acetate and lead citrate. La^{3+} perfusions were performed as in [15]: sections were left unstained.

3. Results and discussion

Enzymic radioiodination is used in this study to selectively label the externally exposed membrane proteins of the two plasma membranes of the cultured epithelial cells. The feasibility of this approach lies in the unique properties of the cultured epithelial monolayers. Under the conditions of growth employed, the cultured cells form a continuous single-cell thick monolayer with apical tight junctions limiting diffusion between the cells (fig.1A). The impermeability of the cell monolayer to lactoperoxidase can be inferred from the data given in table 1 giving a transmonolayer resistance of $\simeq 4\ k\Omega.cm^{-2}$ similar to 'tight' epithelia such as frog skin and mammalian collecting ducts [16]. Junctional permeability to large molecules such as lactoperoxidase under these conditions will be insignificant [16]. Confirmation of this view is given in fig.1C which demonstrates that when the electron dense tracer molecule La^{3+} is perfused from the apical (or brush-border) surface little or no penetration of La^{3+} occurs through the tight-junction.

Table 1
Active transport potentials and monolayer electrical resistance measured in Ussing chambers in symmetrical Krebs solutions [11]

n	Potential difference (mV)	Resistance ($k\Omega.cm^{-2}$)
12	+3.12 ± 1.57	4.135 ± 1.247

Electrical potential difference is expressed in relation to the basal (basal-lateral) bathing solutions. Errors ± SEM

Asymmetry in the distribution of externally exposed proteins is to be expected on the basis of the maintenance of an active ion transport potential difference (table 1), the known asymmetry in the distribution of the Na-K ATPase in this cell-line [10] and to the ultrastructural differences observed between the apical brush-border membrane, which shows an extensive glycocalyx, and the basal-lateral membrane, which is devoid of such projections (fig.1B).

The electrophoregrams shown in fig.2 convincingly demonstrate the preferential location of a number of externally exposed iodinated membrane proteins to either the apical or basal-lateral membranes. There is little homology between the iodination patterns of the exposed apical and basal-lateral membrane proteins, both of which are distinct from the total

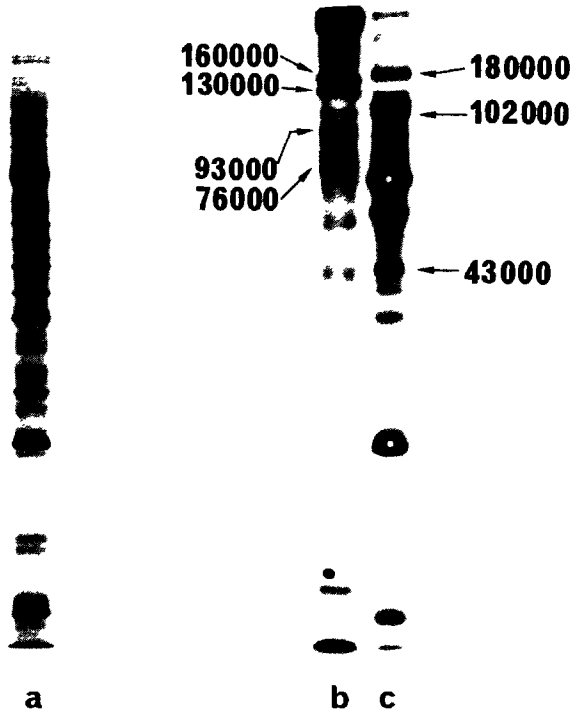

Fig.2. Electrophoregrams of MDCK cell monolayers. (a) Coomassie brilliant blue-stained electrophoretic pattern of whole cells. (b) Autoradiograph of apical-iodinated externally-exposed membrane proteins (c) Autoradiograph of basal-iodinated externally-exposed membrane proteins. White dots indicate serum proteins absorbed on to the basal surface of the cells. Numbers indicate molecular weight estimates.

cell protein (Coomassie brilliant blue pattern). The majority of proteins detected on the apical surface are located solely on this surface. The majority of proteins found on the basal-lateral surface are restricted to this surface. Thus the external surfaces of the apical and basal-lateral membranes appear to be very discrete membrane domains.

Identification of the major iodinated membrane proteins is not possible on present evidence alone. However, there are similarities between the major bands observed in this study as compared with the known composition of mammalian kidney cortical brush-border microvillus proteins [17,18]. Major membrane protein bands in kidney microvilli occur at app. mol. wt: 160 000 (aminopeptidase M); 130 000 (dipeptidylpeptidase); 95 000 (neutral endopeptidase); and 80 000 (alkaline phosphatase) [17,18]. Similar molecular weight iodinated protein bands in the brush-border of MDCK cells occur at 160 000, 130 000, 93 000 and 76 000. The large extrinsic glycoprotein of 180 000 mol. wt observed [18] has no homologous band in the autoradiographic pattern of MDCK brush-borders. In respect of the basal-lateral proteins there are apparent homologies between the iodination pattern and the known molecular weight of the Na-K ATPase which consists of a large polypeptide of $\simeq$ 95 000 mol. wt and a small glycoprotein of $\simeq$ 45 000 mol. wt [19] and to the LETS protein ($\simeq$ 200 000 mol. wt) which is known to be important in cell substrate attachment in various cultured cell-lines [20]. In MDCK cells similar molecular weight iodinated protein bands occur at 102 000, 43 000 and 180 000.

The demonstration of protein asymmetries in the MDCK epithelium should establish this cell-line as a useful culture model to study the factors controlling the formation and maintenance of asymmetrical protein compositions in epithelial cells.

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