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Uteroglobin, an apically secreted protein of the uterine epithelium, is secreted non-polarized from MDCK cells and mainly basolaterally from Caco-2 cells

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A complete cDNA encoding rabbit uteroglobin was constructed and expressed in MDCK and Caco-2 cells. The MDCK cells secrete uteroglobin in approximately equal amounts to the apical and the basolateral side, whereas the Caco-2 cells secrete uteroglobin mainly to the basolateral side. Both MDCK and Caco-2 cells thus secrete uteroglobin in a non-sorted manner. It has, however, previously been shown that uteroglobin is secreted exclusively at the apical membrane in primary cell culture of endometrial epithelial cells [S.K. Mani et al. (1991) Endocrinology 128, 1563–1573]. This suggests that either the endometrial epithelium has an apical default pathway or recognises a sorting signal not recognised by MDCK cells and Caco-2 cells. Our data thus show that a soluble molecule can be secreted at the apical, the basolateral or both membranes depending on the cell type.

Uteroglobin; MDCK; Caco-2; Sorting; Non-polarized; Aminopeptidase N

1. INTRODUCTION

The plasma membrane of epithelial cells is divided into an apical and a basolateral domain by tight junctions. Epithelial cells are able to secrete proteins in a polarized fashion at either of these membrane domains. Thus, the MDCK cell line secretes gp 80 [1], osteopontin-related polypeptide [2] and plasminogen activator [3] at the apical membrane and laminin and heparan sulphate proteoglycan at the basolateral membrane [4]. Proteins normally absent from the cell type investigated have, after transfection, been used as non-sorted molecules, assuming that such proteins lack features important for sorting. Sorting to either of the two destinations requires a specific sorting event in MDCK-cells, since various presumably non-sorted molecules are secreted in approximately equal portions at the two membrane domains [5–8]. Efforts aiming at the identification of the molecular determinant on membrane proteins responsible for the apical targeting, using mutagenesis, have mapped the apical sorting signal to the ectodomain of three different mammalian trans-membrane proteins [7–9]. However, these ectodomains are large and difficult to further analyse by mutagenesis. A small apically secreted protein with a known three-dimensional structure would constitute a good model for mapping of the apical sorting signal in more detail. The availability of such a protein would allow the selective mutagenesis of surface-exposed amino acids, without interfering with the folding of the molecule. Uteroglobin fulfils these criteria and we have therefore initiated studies on this 70 amino acid protein. Uteroglobin is secreted exclusively at the apical membrane of primary cell cultures of endometrial epithelial cells after they have been allowed to form a tight monolayer in vitro [10]. Immunocytochemistry and in situ hybridization studies have shown that uteroglobin is also expressed in several other epithelial cell types [11–15] and the three-dimensional structure has been determined [16].

We report that uteroglobin is secreted in a non-sorted manner; non-polarized from MDCK cells and mainly basolaterally from Caco-2 cells. Both MDCK and Caco-2 cells thus differ from endometrial cells, where uteroglobin is secreted exclusively apically. These observations suggest that either the endometrial cells have an apical default pathway or that the MDCK and Caco-2 cells are not able to recognize some apical sorting signals recognized by the endometrial cells.

2. MATERIALS AND METHODS

2.1. *Cell culture and DNA constructions*

MDCK cells stain II (a gift from K. Mostov, University of California, San Francisco) and Caco-2 cells (a gift from M. Spiess, Biocenter, Basel) were grown in minimal essential medium supplemented with 2 mM L-glutamine, 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The MDCK and Caco-2 cells were transfected as described

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[17] for adherent cells in suspension. The transfected cells were selected using G418 and stable clones were isolated using cloning cylinders.

Using PCR a fragment coding for the two last amino acids of the signal peptide and for the entire coding region of uteroglobin was amplified from a partial rabbit uteroglobin cDNA [18] using a 3′ primer containing an additional *Bam*HI site This fragment was subcloned into *Pst*I–*Bam*HI cleaved pBluescript SK$^+$ Also, using PCR the first exon was amplified from the cloned rabbit uteroglobin gene [19] using a 3′ primer extended by a *Sal*I site and 5′ primer extended by the coding region for the last 3 amino acids of the signal peptide The 5′ primer contained in addition a silent mutation deleting the *Pst*I site in the signal sequence This fragment was inserted in front of the uteroglobin coding sequence and the construct was sequenced to confirm the entire coding region The complete coding region for the rabbit uteroglobin was then subcloned into the eukaryotic expression vector pTEJ-4 [20]. The construction of the secretory anchor/stalk minus aminopeptidase N cDNA was previously described [21]. Cells were transfected with either 20 μg of pTEJ-4 containing the entire coding sequence of uteroglobin and 2 μg pSV2-neo [22] or cotransfected with 10 μg pTEJ-4 containing the entire coding sequence of uteroglobin, 10 μg pTEJ-4 containing the anchor/stalk minus aminopeptidase N cDNA and 2 μg pSV2-neo

2.2 *Analytical methods*

Cells (10^6) per well were seeded into 24-mm Transwell chambers (Costar Europe Ltd. Badhoevedorp, The Netherlands) The medium was changed daily Tightness of filter-grown monolayers was assayed by filling the inner chamber to the brim and allowing it to equilibrate overnight [23]. On day 3 the cells were washed once in minimal essential medium lacking methionine but containing 10% dialyzed FCS (methionine-free media) and then incubated for 30 min in methionine-free media (1 ml/well). 10 μCi [^{35}S]methionine were then added to each well and followed by incubation for 30 min. When indicated the media were changed to complete media and the incubation was continued at 37°C for the designated chase period. The apical and basolateral media were collected and 200 μl proteinase inhibitor buffer (PBS containing 0 1% bovine serum albumin, 1 mM EDTA, 2 μg/μl aprotinin) was added to each sample. For immunoprecipitation the sample was added 1 μl of sheep antiserum raised against human uteroglobin, incubated 1 h at 4°C followed by the addition of 3 μl rabbit anti-sheep immunoglobulin (Dakopatts Z138) and a further 1 h incubation at 4°C. The immunoprecipitate was collected on Protein A-Sepharose and washed 3 times in proteinase inhibitor buffer before it was analyzed on 18% SDS-PAGE The gels were finally dried and processed for densitometry As molecular weight markers myoglobin (17 kDa), myoglobin I + II (14 kDa) and myoglobin I + III (11 kDa) were used.

For enzymatic analysis the apical and basolateral media were collected and assayed for its aminopeptidase N activity [24] using the substrate L-alanine-*p*-nitroanilide

3. RESULTS AND DISCUSSION

MDCK and Caco-2 cells were transfected with an expressing vector carrying cDNA coding for uteroglobin. Clones were screened for uteroglobin expression using metabolic labelling with [^{35}S]methionine followed by immunoprecipitation and analysis by SDS-PAGE. Several clones of both MDCK and Caco-2 cells expressing uteroglobin were identified. Transfected and non-transfected MDCK cells and Caco-2 cells were labelled with [^{35}S]methionine for 30 min and chased for 5 h. The media were collected, immunoprecipitated and analyzed by SDS-PAGE. The media from transfected MDCK cells (Fig. 1, lane 1) and Caco-2 cells (lane 3) contained uteroglobin whereas the media from non-transfected MDCK (lane 2) and Caco-2 cells (lane 4) did not.

In order to investigate the partition of uteroglobin between the apical and the basolateral domains, transfected MDCK and Caco-2 cells were grown on filters until a tight confluent monolayer was formed. The cells were labelled with [^{35}S]methionine for 30 min, chased for 2 h and the uteroglobin in the media was immunoprecipitated and quantitated. When MDCK cells were investigated (Fig. 2) uteroglobin was found in approximately equal amounts in the apical and the basolateral media 46 ± 4% of the total uteroglobin secreted was secreted to the apical side and 54 ± 4% to the basolateral side (three independent clones were investigated with similar results). Transfected Caco-2 cells secreted 28 ± 13% of the secreted uteroglobin to the apical side and 72 ± 13% to the basolateral side (Fig. 2).

Recombinant expression of various proteins have previously been analyzed to investigate the signal-independent vesicular bulk transport in MDCK cells [5–8]. In all cases non-sorted proteins were found to be secreted from MDCK cells in approximately equal amounts at the two membrane domains. Uteroglobin is thus secreted from MDCK cells in a non-sorted manner.

In Caco-2 cells recombinant expressed non-sorted proteins are secreted exclusively [25] or predominantly to the basolateral side [26]. The present result thus indicates that also Caco-2 cells secretes uteroglobin in a signal-independent way. It was recently shown that the urokinase-type plasminogen activator is secreted mainly at the apical membrane of MDCK cells but preferentially at the basolateral membrane of Caco-2 cells [3]. In addition Caco-2 cells release all of their endogenous secretory proteins preferentially to the basolateral side [25]. One possible interpretation of these observations is that Caco-2 cells are devoid of an apical pathway for secretory proteins, and consequently uteroglobin as well is secreted to the basolateral side. In contrast MDCK cells are able to specifically target secretory proteins to the apical side [1–3].

Fig 1. Recombinant expression of uteroglobin. MDCK or Caco-2 cells, transfected and non transfected, were grown on filters and pulse-labelled with [^{35}S]methionine for 30 min followed by a 5 h chase. The media were collected and immunoprecipitated and analyzed by SDS-PAGE Transfected MDCK cells (lane 1), non-transfected MDCK cells (lane 2), transfected Caco-2 cells (lane 3) and non-transfected Caco-2 cells (lane 4) are shown. The positions of marker proteins and their molecular masses in kDa are indicated

It has been reported that uteroglobin in the presence of progesterone is taken up by the endometrium [28]. The possibility that also MDCK cells take up uteroglobin possibly followed by transcytosis was therefore investigated. MDCK cells expressing uteroglobin were labelled with [^{35}S]methionine for 30 min and chased for 2 h. The media, containing metabolically labelled uteroglobin were collected, and applied to one side of filter grown non-transfected monolayers of MDCK cells and after a 6 h incubation the media from both sides were collected and immunoprecipitated. Less than 5% of the uteroglobin applied to the apical side was found on the basolateral side and less than 5% of the uteroglobin that was applied to the basolateral side was found on the apical side. Transcytosis is thus negligible under the conditions used.

In order to verify that the clones investigated have not lost their ability to secrete a protein to the apical side, MDCK cells were cotransfected with an expression vector carrying cDNA coding for uteroglobin and an expression vector carrying cDNA coding for anchor/stalk minus aminopeptidase N. We have previously shown that anchor/stalk minus aminopeptidase N is secreted to the apical side of MDCK cells [21]. Two clones expressing both uteroglobin and anchor/stalk minus aminopeptidase N were identified. One clone was grown to confluence on filters and labelled with [^{35}S]methionine for 30 min and then chased for 2 h. The apical and basolateral media were collected and analyzed for aminopeptidase N activity and the uteroglobin was immunoprecipitated and quantitated by densitometry. By enzymatic analysis anchor/stalk minus aminopeptidase N was found to be secreted 82 ± 5% of the total activity secreted to the apical side and 18 ± 5% to the basolateral side. This is similar to previous findings [21] and to the distribution of membrane-bound aminopeptidase N in MDCK cells [29]. Uteroglobin was secreted 52 ± 6% of the total secreted uteroglobin to the apical side and 48 ± 6% to the basolateral side. The clones investigated have thus not lost the ability to sort and target to the apical side.

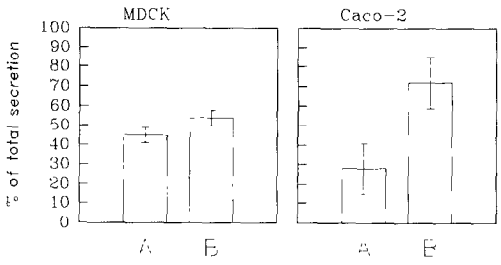

Fig. 2. Secretion of uteroglobin from MDCK and Caco-2 cells. Transfected MDCK or Caco-2 cells were grown to confluence on filters, labelled with [^{35}S]methionine for 30 min and chased for 2 h. Apical (A) and basolateral (B) media were collected, immunoprecipitated and analyzed by SDS-PAGE and autoradiography The mean and standard deviation of three experiments are shown

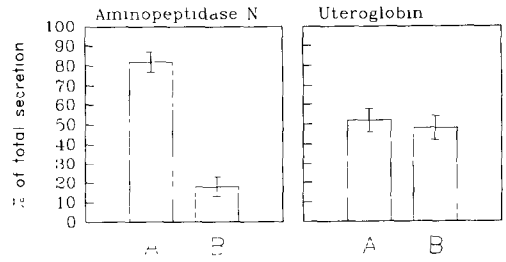

Fig 3. MDCK cells are able to sort anchor/stalk minus aminopeptidase N but not uteroglobin. MDCK cells coexpressing anchor/stalk minus aminopeptidase N and uteroglobin were labelled with [^{35}S]methionine for 30 min and chased for 2 h. Apical (A) and basolateral (B) media were collected and analyzed for aminopeptidase N activity and uteroglobin using immunoprecipitation, SDS-PAGE and autoradiography. The mean and standard deviation of three experiments are shown.

The results presented here show that uteroglobin is secreted in a signal-independent manner from both MDCK and Caco-2 cells. Primary cell cultures of endometrium epithelium secrete uteroglobin exclusively apically probably because this particular cell type has an apical default pathway. Alternatively, it is possible that the endometrial cells and maybe other epithelial cell types recognize and act on other sorting signals than those recognized in the MDCK cell type. It is not likely that the differences observed are due to species differences as the MDCK cells are able to sort and transport other murine proteins to the apical membrane [30].

Our data thus show that a soluble molecule can be secreted at the apical, the basolateral, or both membranes depending on the cell type. Also the LDL-receptor is located in a tissue-specific manner when expressed in transgenic mice [31]: apical in kidney cells and basolateral in liver and intestine. Likewise a viral glycoprotein, p62/E2, is routed differently in the two polarized cell lines FRT and Caco-2 [32]: apical in the FRT cells and basolateral in the Caco-2 cells. Whether these differences are also a result of cell type specific default pathways or a result of the existence of multiple sorting mechanisms operating in different cell types, remains to be settled.

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