Polarized secretion of apoA-I and apoA-II by transfected MDCK cells

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Abstract Apolipoproteins (apo) are secreted preferentially from the basolateral surface of hepatocytes and enterocytes. The polarized secretion of proteins is either mediated by a protein-dependent sorting signal or by a cell-dependent default pathway. In order to determine the mechanism for the polarized secretion of apolipoproteins, we examined the secretion of apoA-I and apoA-II in transfected Madin-Darby canine kidney (MDCK) cells. Transfected MDCK cells and Caco-2 cells were grown as a polarized monolayer on tissue culture inserts, which separate an upper apical compartment from the lower basolateral compartment, and the secretion of apoA-I and apoA-II into the apical and basolateral compartments was quantitated by immunoprecipitation. Caco-2 cells almost exclusively secreted apoA-I and apoA-II basolaterally, with an apical to basolateral ratio of 18:82 for apoA-I, and 11:89 for apoA-II. In contrast, transfected MDCK cells secreted significant amounts of apoA-I and apoA-II into both compartments, but with a bias toward apical secretion and an apical to basolateral ratio of 66:34 and 68:32, respectively. The polarized secretion of MDCK cells was not due to transcytosis, diffusion, or differential recovery. As assessed by density gradient ultracentrifugation, apoA-I and apoA-II secreted from either the apical or basolateral surface were relatively lipid-poor. Overall, these results suggest that the polarized secretion of apoA-I and apoA-II does not occur by a protein-dependent sorting signal, but by a cell-dependent default pathway that leads to preferential basolateral secretion by Caco-2 cells and both apical and basolateral secretion in MDCK cells, but with a bias toward apical secretion.—**Remaley, A. T., and J. M. Hoeg.** Polarized secretion of apoA-I and apoA-II by transfected MDCK cells. *J. Lipid Res.* 1995. **36:** 407–413.

Supplementary key words MDCK cells • apical secretion • basolateral secretion • apolipoproteins

The apical and basolateral membranes of polarized cells differ in their protein composition due to the polarized secretion of membrane-bound proteins (1–3). In addition to membrane-bound proteins, secretory proteins are secreted in a polarized manner (4–9). The polarized secretion of proteins can occur by a protein-dependent sorting signal or by a cell-dependent default pathway (1–3). Protein-dependent secretion depends on the presence of sorting signals on proteins that specifically direct the secretion of proteins to either the apical or basolateral side. Both apical and basolateral sorting signals have been identified (10–14). One class of basolaterally secreted membrane-bound proteins, which include the LDL receptor (12), contains sorting signal domains in the cytoplasmic tail (10–12). In contrast, many apically directed membrane-bound proteins are covalently modified by glycosylphosphatidylinositol linkages, which can serve as apical sorting signals (13, 14). Proteins that do not contain sorting signals are believed to be secreted via a default pathway, which in a cell-dependent manner can lead to either apical or basolateral secretion (4, 5, 15, 16).

The principal cells that secrete apolipoproteins, hepatocytes and enterocytes, are polarized cells, with two distinct plasma membranes separated by tight junctions (2). The apical membrane of the hepatocyte communicates with the biliary system, and the basolateral membrane faces the hepatic sinusoids. The apical membrane of the enterocyte surrounds the lumen of the intestine, whereas the basolateral membrane communicates with the lymphatic and circulatory system. Apolipoproteins, along with most other plasma proteins, are preferentially secreted by hepatocytes toward the basolateral direction into the perisinusoidal space (6). Similarly, enterocytes primarily secrete apolipoproteins basolaterally (4, 5).

A likely mechanism for the polarized secretion of apolipoproteins is that it occurs by a default pathway, which for hepatocytes and enterocytes is the basolateral route (1–3, 5, 15). This simple model, however, may not fit for all secretory proteins (1–3), including apolipoproteins. Some proteins that follow the default pathway in heptocytes and enterocytes still contain a protein-dependent sorting signal, as determined by heterologous expression in another cell line with a different default pathway (10, 11, 17). This redundancy in mechanisms for secretion may

Abbreviations: apo, apolipoprotein; MDCK, Madin-Darby canine kidney cells; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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ensure the proper orientation of secretion of some proteins. Furthermore, although the majority of plasma proteins synthesized by hepatocytes are secreted basolaterally via the default pathway by hepatocytes (1–3, 6), a small fraction of albumin has been shown to be directly secreted into the bile through the apical plasma membrane domain (18). ApoA-I has also been detected in small amounts in the bile, where it may play a role in preventing gallstone formation (19). Mucosal organ explants of the small intestine have also been shown to secrete apoA-I apically, and the degree of apical secretion has been suggested to be modulated by the availability of lipid to associate with apoA-I and form lipoprotein particles (20). Whether the limited apical secretion of apoA-I in hepatocytes and enterocytes occurs directly or indirectly, by paracellular transport or transocytosis (1–3, 6, 18) of basolaterally secreted apoA-I is not known. Interestingly, apoJ, which follows the default basolateral pathway in hepatocytes (21–24), is almost exclusively secreted into the apical compartment, when endogenously synthesized by kidney tubular cells (21–24). The default pathway in kidney tubular cells normally leads to secretion into both the apical and basolateral compartment (25–27). The differential ability of apoJ to associate with lipid between the two cell types has also been proposed to account for the difference in the orientation of secretion (2). Overall, these results suggest that the mechanism of polarized secretion of apolipoproteins may be more complex than a simple default pathway model.

An experimental approach for analyzing the polarity of secretion of proteins is to culture epithelial cells in vitro on a permeable membrane support (1–3). When grown as a monolayer on a membrane support, which separates the upper apical compartment from the bottom basolateral compartment, epithelial cells become morphologically and functionally polarized. The enterocytic Caco-2 cell line, when grown as a polarized monolayer, preferentially secretes apolipoproteins to the basolateral side (4, 5). Madin-Darby canine kidney (MDCK) (28) cells, a renal tubular cell line, can also be grown as a polarized monolayer and have been commonly used for heterologous expression of proteins, in order to compare the orientation of secretion of the expressed protein to its orientation in a native cell type. This approach combined with site-directed mutagenesis has been used to identify not only proteins that contain sorting signals but also the location of sorting signals on proteins.

We examined the mechanism for the polarized secretion of apoA-I and apoA-II, the major proteins of high density lipoproteins (29, 30), by comparing the secretion by Caco-2 cells of endogenously produced apoA-I and apoA-II to heterologously produced apoA-I and apoA-II produced by transfected MDCK cells. Caco-2 cells primarily secreted apoA-I basolaterally, as previously described (4, 5) as well as apoA-II, whereas transfected MDCK cells secreted apoA-I and apoA-II into both compartments, but with a bias toward apical secretion. These results suggest that the polarized secretion of apoA-I and apoA-II is unlikely to be mediated by a protein-dependent sorting signal but instead depends on a cell-dependent default pathway, which differs between Caco-2 cells and MDCK for apolipoproteins.

METHODS

Cell culture

MDCK II cells (28) and Caco-2 cells (American Type Culture Collection) were grown in alpha-modified Minimum Essential Medium (GIBCO BRL) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B. For studies on the polarity of secretion, cells were grown on Anopore membrane tissue culture inserts (Nunc), which contain a membrane that supports the cell monolayer and separates the apical (upper) from the basolateral (lower) compartment. The integrity and confluence of the cell monolayer was assessed morphologically by light microscopy and by transepithelial resistance with a voltohmmeter (World Precision Instruments).

Plasmid construction and transfection

A cDNA for apoA-I (29) was ligated into the unique BamHI site on the pLen expression vector (31) to produce a plasmid designated pLenA-I. A partial cDNA for apoA-II (30) was extended by the polymerase chain reaction with an oligonucleotide that coded for the missing amino acid residues from −1 to −12 and ligated into the BamHI site of the pLen plasmid to produce a plasmid designated pLenA-II. The sequence of the cDNA for apoA-I and apoA-II was confirmed by dideoxy DNA sequencing. Subconfluent MDCK II cells were co-transfected with either 25 μg of pLenA-I or pLenA-II and 1 μg of pSV2neo (32) by a calcium phosphate precipitation method (33). At 48 h, the medium was replaced with fresh medium containing 1 mg/ml of active G-418, and thereafter the medium was replaced every 2 days with fresh medium containing G-418. At 14 days, G-418-resistant colonies were isolated, re-cultured, and screened for expression of apoA-I and apoA-II by immunoprecipitation, as described below. Clonal cell lines were isolated by limiting dilution.

Quantitation of apolipoprotein secretion

Cells were metabolically labeled for 2 h with 1.5 ml of 100 μCi/ml of Trans[^{35}S]-label (ICN) in methionine and cysteine-free Dulbecco's Modified Eagle Medium (GIBCO BRL) placed in the basolateral compartment, supplemented with 2 mM glutamine, 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of

amphotericin B. An equal volume of media without Trans[^{35}S]-label was placed in the apical compartment, and results were analyzed only for those wells that did not have any net exchange of media between the two compartments during the 2-h period. Equal aliquots of media from the apical and basolateral compartment were then immunoprecipitated with either polyclonal sheep anti-apoA-I or anti-apoA-II antibody (Boehringer Mannheim) and Pansorbin (CalBiochem) as previously described (34). Immunoprecipitated apolipoproteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions on a 10–20% gradient tricine gel (Novex) and autoradiography was performed after treatment with Amplify (Amersham). Results from autoradiography were quantitated by densitometry, and pairwise comparisons between the apical and basolateral secretion were analyzed by the *t*-test. Equal exposure times for the autoradiograms were used when comparing the results of the apical and basolateral compartment. The apical to basolateral ratios for secretion of apoA-I and apoA-II were determined from the percent of protein present in each compartment.

Density gradient ultracentrifugation

Density gradient ultracentrifugation was performed on media from cells labeled with Trans[^{35}S]-label and supplemented with PMSF (100 μg/ml), pepstatin (1 μg/ml), leupeptin (1 μg/ml), and aprotinin (1 μg/ml). Cell-culture media were adjusted to a density of 1.35 g/ml with KBr, and continuous density gradient ultracentrifugation was performed (35). Fractions of 1.2 ml were collected, and density was determined gravimetrically. Apolipoproteins were immunoprecipitated from the fractions after dialysis in phosphate-buffered saline.

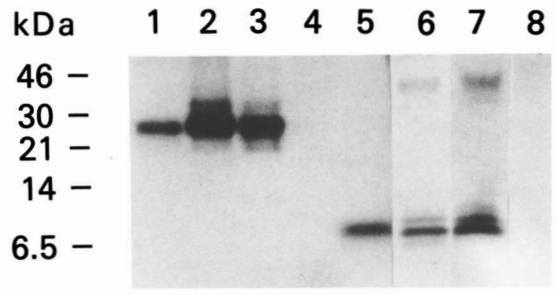

Fig. 1. Expression of apoA-I and apoA-II by MDCK cells. Media from the following cell lines metabolically labeled with Trans[^{35}S]-label or radioiodinated apolipoproteins were immunoprecipitated for either apoA-I (lanes 1–4) or apoA-II (lanes 5–8) and analyzed by SDS-PAGE, followed by autoradiography: ^{125}I-labeled apoA-I purified from plasma (lane 1), apoA-I from Caco-2 cells (lane 2), apoA-I from transfected MDCK cell media (lane 3), apoA-I from non-transfected MDCK cells (lane 4), ^{125}I-labeled apoA-II purified from plasma (lane 5), apoA-II from Caco-2 cells (lane 6), apoA-II from transfected MDCK cells (lane 7), and apoA-II from non-transfected MDCK cells (lane 8).

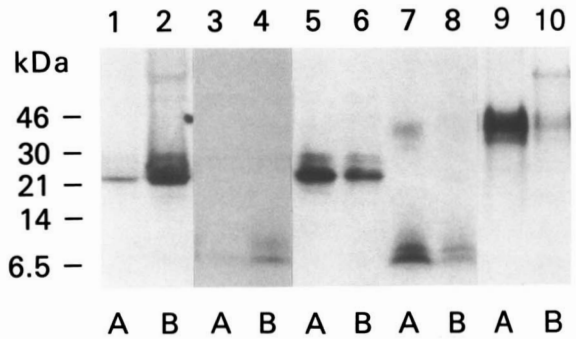

Fig. 2. Polarized secretion of apoA-I and apoA-II. Cells were grown on tissue culture inserts and metabolically labeled with Trans[^{35}S]label for 2 h. Media from the apical (A) and basolateral (B) compartments of Caco-2 cells (lanes 1–4) and transfected MDCK cells (lanes 5–8) were immunoprecipitated for either apoA-I (lanes 1, 2, 5, and 6) or apoA-II (lanes 3, 4, 7, and 8) and analyzed by SDS-PAGE, followed by autoradiography. Media were analyzed for total protein secretion from the apical (lane 9) and basolateral (lane 10) compartments of MDCK cells by trichloroacetic acid precipitation, followed by SDS-PAGE, and autoradiography.

RESULTS

Expression of apoA-I and apoA-II by MDCK cells

Stable cell lines of MDCK cells expressing apoA-I and apoA-II were produced by transfection with the pLenA-I and pLenA-II expression plasmid, as described in the Methods section. Transfected MDCK cells were metabolically labeled with Trans[^{35}S]-label, and media were immunoprecipitated for either apoA-I or apoA-II and analyzed by SDS-PAGE and autoradiography (**Fig. 1**). Transfected MDCK cells produced apoA-I (lane 3) and apoA-II (lane 7), which co-migrated with apoA-I (lane 2) and apoA-II (lane 6) produced by Caco-2 cells and with standard apoA-I (lane 1) and apoA-II (lane 5) purified from plasma. At least two forms of apoA-II were observed to be secreted by Caco-2 cells and MDCK cells, which has been previously observed in several other cell lines to be due to variable O-linked glycosylation (36, 37). Non-transfected MDCK cells did not secrete detectable amounts of apoA-I (lane 4) or apoA-II (lane 8).

Polarized secretion of apoA-I and apoA-II

In order to examine the polarized secretion of apoA-I and apoA-II, Caco-2 cells and transfected MDCK cells were grown as a polarized monolayer on tissue culture inserts, which contain a membrane that supports the cell monolayer and separates the upper apical and lower basolateral compartments. After reaching confluence and forming a tight monolayer, as determined by transepithelial resistance and microscopic examination, the cells were metabolically labeled with Trans[^{35}S]-label for 2 h. Equal aliquots of media from the apical and basolateral compartments were immunoprecipitated for either apoA-I or apoA-II and analyzed by SDS-PAGE, followed by autoradiography (**Fig. 2**). Caco-2 cells secreted both apoA-I

(lane 1 vs. lane 2) and apoA-II (lane 3 vs. lane 4) almost exclusively into the basolateral compartment. In contrast, MDCK cells secreted apoA-I and apoA-II with the opposite polarity than that of Caco-2 cells. The majority of apoA-I (lane 5 vs. lane 6) and apoA-II (lane 7 vs. lane 8) produced by transfected MDCK cells was secreted apically, although a significant amount of apoA-I and apoA-II was also secreted into the basolateral compartment. The functional polarity of the MDCK cells was shown to be preserved, as assessed by their polarized secretion of the major apical marker secretory protein (lane 9 vs. 10), which has recently been identified as apoJ (21–24).

In **Fig. 3,** we determined the relative percentage of apoA-I and apoA-II that was secreted into each compartment from a mean of six experiments. ApoA-I was secreted by Caco-2 cells with an apical to basolateral ratio of 18:82, whereas transfected MDCK cells secreted apoA-I with an apical to basolateral ratio of 66:34. We found similar results for apoA-II; Caco-2 cells secreted apoA-II with an apical to basolateral ratio of 11:89, and transfected MDCK cells secreted apoA-II with a ratio of 68:32. After forming a monolayer and reaching confluence, the relative percent distribution of apoA-I and apoA-II secreted by the transfected MDCK cells did not significantly change when monitored over a 7-day period (data not shown). All pairwise comparisons between the apical and the basolateral compartments were statistically significant ($P < 0.01$). These results suggest that although apoA-I and apoA-II are secreted in a polarized manner by transfected MDCK cells, they are secreted with a polarity opposite than that of Caco-2 cells, and unlike Caco-2 cells, the polarized secretion by MDCK cells was not as efficient. We detected significant amounts of apoA-I and apoA-II in both compartments, but with a bias toward apical secretion.

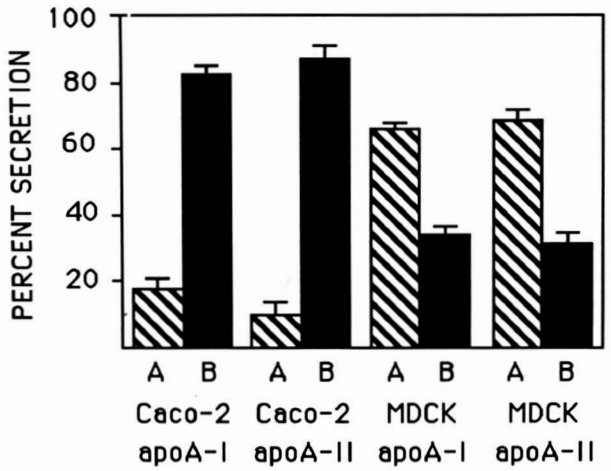

Fig. 3. Relative percent secretion of apoA-I and apoA-II. The relative percent secretion of apoA-I and apoA-II into the apical (A, diagonal bars) or basolateral (B, solid bars) compartments was quantitated by immunoprecipitation and densitometry for Caco-2 cells and transfected MDCK cells, as indicated. Results are shown as the mean ± the standard error of the mean (n = 6).

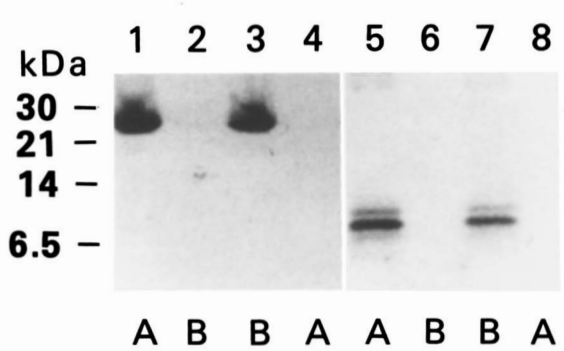

Fig. 4. Permeability of MDCK monolayers. Conditioned media from cells metabolically labeled with Trans[^{35}S]-label from apoA-I transfected MDCK cells were added to either the apical (lanes 1) or basolateral (lanes 3) compartment of non-transfected MDCK cells grown on tissue culture inserts. Similarly, conditioned media from cells metabolically labeled with Trans[^{35}S]-label from apoA-II transfected MDCK cells were added to either the apical (lanes 5) or basolateral (lanes 7) compartment of non-transfected MDCK cells grown on tissue culture inserts. After a 2-h incubation, the media from the apical (A) and basolateral (B) compartments were immunoprecipitated for either apoA-I (lanes 1–4) or apoA-II (lanes 5–8).

We next assessed whether apoA-I or apoA-II was initially secreted by MDCK cells into the basolateral compartment and then later transferred during the 2-h labeling period to the apical compartment by transocytosis, as occurs for other proteins (1–3). Conditioned media from transfected MDCK cells metabolically labeled with Trans[^{35}S]-label were added to either the apical or the basolateral compartment of non-transfected MDCK cells grown on tissue culture inserts. The transfer of radiolabeled apoA-I and apoA-II across the cell monolayer to the other compartment was monitored 2 h later by immunoprecipitation of media from both compartments. Whether the radiolabeled apoA-I or apoA-II was added to either the basolateral or apical compartment, there was no detectable transfer of either protein to the other compartment (**Fig. 4**). This excludes the possibility of significant transcytosis or diffusion of apoA-I or apoA-II across the MDCK cell monolayer during the 2-h period of the experiment described in Fig. 2. In addition, we had nearly equal and full recovery (over 85%) of the added radiolabeled apoA-I and apoA-II from both the apical and basolateral compartments, thus excluding differential recovery of the protein as the explanation for the asymmetric secretion.

Density distribution of apolipoproteins secreted by MDCK cells

Because the association of proteins to lipids has been shown to affect the polarity of secretion (13, 14), we examined the association of apoA-I and apoA-II with lipid (**Fig. 5**). Regardless of the orientation of secretion, both

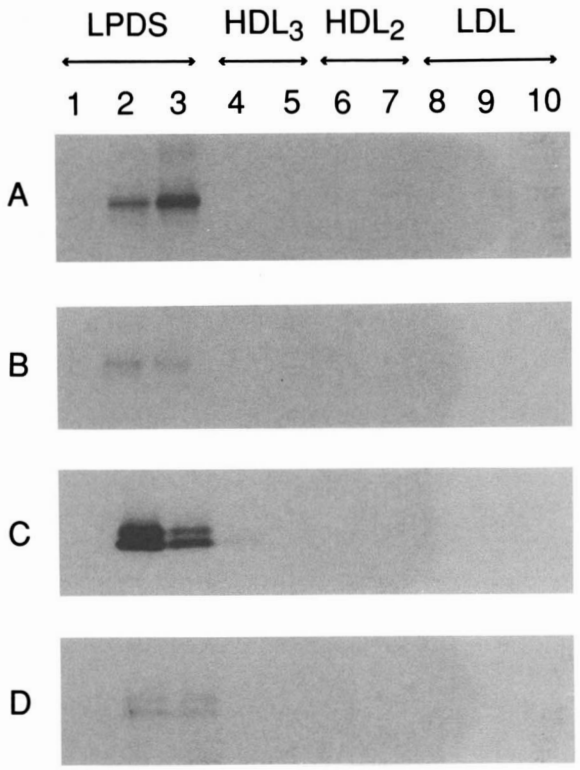

Fig. 5. Density distribution of apoA-I and apoA-II secreted by MDCK cells. Media from transfected MDCK cells labeled with Trans[^{35}S]-label were separated by continuous density gradient ultracentrifugation, immunoprecipitated, and analyzed by SDS-PAGE, followed by autoradiography for the following samples: apical secreted apoA-I (panel A), basolateral secreted apoA-I (panel B), apical secreted apoA-II (panel C), and basolateral secreted apoA-II (panel D). Based on the density of each fraction, the arrows above each lane indicate the approximate location of the major lipoprotein density fractions.

apoA-I and apoA-II were relatively lipid-poor. Both apoA-I from the apical (panel A) and basolateral (panel B) compartments and apoA-II from the apical (panel C) and basolateral (panel D) compartments were found by density gradient ultracentrifugation to be relatively lipid-poor. This result is similar to previous studies of several other transfected cells, which readily secrete apoA-I and apoA-II in the absence of significant amounts of bound lipid (36, 38).

DISCUSSION

Basolateral secretion was initially proposed to be the default pathway for secretion in polarized cells, and apical secretion was proposed to be mediated by protein-dependent sorting signals (1–3). This was hypothesized because the apical surface of epithelial cells contain unique proteins not present on other cell types. This appears to be true for hepatocytes, which almost exclusively secrete both membrane-bound proteins and secretory proteins basolaterally (1–3, 6). Most apically directed hepatic proteins are initially secreted basolaterally and then transferred to the apical side by transcytosis, which also depends on the presence of a sorting signal (1–3). This pathway is also present in enterocytes, although enterocytes have a limited ability to directly secrete proteins to the apical surface (5, 8, 9, 15). In contrast, MDCK cells can directly secrete proteins to both the apical and basolateral surface (7, 16), and the sorting of secretory vesicles destined for secretion at the apical or basolateral surface occurs at the *trans*-Golgi network (1–3).

Although the basolateral route appears to be the default pathway in some cells, many basolaterally secreted proteins also contain protein-dependent sorting signals that facilitate their polarized secretion (10, 11, 17). Redundancy in the mechanisms for polarized secretion may ensure the proper orientation of secretion of some proteins. Basolateral sorting signals have been identified in the cytoplasmic tails of several membrane-bound proteins (1, 10, 11), including the LDL-receptor (12). One class of apically secreted proteins has been shown to be covalently modified by glycosylphosphatidylinositol linkages, which serve as an apical sorting signal (13, 14). Sorting signals on secretory proteins have not been definitively identified but presumably result in the selective interaction of the secretory protein with some component on either the apical or basolateral secretory vesicle membrane. In support of this concept, several apically secreted membrane-bound proteins have been shown to contain an apical sorting signal in their ectodomains (2, 25, 26), which have also been proposed to interact with the components of the secretory vesicle membrane (1–3).

We examined the mechanism for the polarized secretion of apoA-I and apoA-II by expressing these proteins in MDCK cells (Fig. 1), a polarized renal tubular cell line, and by comparing the orientation of secretion of these proteins produced by transfected MDCK cells and Caco-2 cells. We observed that both apoA-I and apoA-II were secreted by MDCK cells with a polarity opposite than that of Caco-2 cells. ApoA-I and apoA-II were primarily secreted basolaterally by CaCo-2 cells and apically by MDCK cells (Figs. 2 and 3). These results could not be explained by transcytosis, diffusion, or differential recovery of the proteins (Fig. 4). Based on the opposite orientation of secretion by Caco-2 cells and MDCK cells, it is also unlikely that the polarized secretion is mediated by the presence of a protein-dependent sorting signal. If the basolateral secretion of apoA-I and apoA-II in Caco-2 cells was mediated by a protein-dependent sorting signal, then the same orientation of secretion would be expected to occur in transfected MDCK cells. MDCK cells have been shown to readily recognize basolateral sorting signals from heterologous proteins normally synthesized by hepatocytes and enterocytes (1–3, 10, 11, 12, 17). If the secretion of apoA-I and apoA-II by MDCK cells occurred

by a simple default pathway, one would expect secretion of proteins into both the apical and basolateral compartment. Furthermore, for the majority of proteins that are secreted by a default route in MDCK cells, there is a bias for basolateral secretion, with a typical apical to basolateral ratio of 40:60 (7, 16, 25–27). Although we detected a significant amount of apoA-I and apoA-II in both compartments, which may be mediated by default secretion, we observed an apical not a basolateral bias in secretion (Fig. 3). This suggests that there may be an additional mechanism, besides the default pathway, for apolipoprotein secretion that leads to an apical bias in MDCK cells for apoA-I and apoA-II. One possibility is that apoA-I and apoA-II contain a cryptic apical protein-dependent sorting signal that is not recognized by Caco-2 cells but is inefficiently recognized by MDCK cells. This is unlikely because MDCK cells and Caco-2 cells have been shown to readily recognize sorting signals present on proteins produced by either cell type (1–3, 10, 11, 12, 17). The other possibility is that the bias for apical secretion may be due to the inherent lipid-binding properties of apolipoproteins. Apical secretory vesicle membranes have been shown to be relatively enriched in cholesterol, phosphatidylethanolamine and glycolipids compared to basolateral secretory vesicle membranes (3). An increased affinity of apolipoproteins to the lipid components of apical secretory vesicles could possibly lead to enrichment of apolipoproteins in these vesicles and to a bias for apical secretion. A similar mechanism accounts for the efficient polarized apical secretion of glycosylphosphatidylinositol-linked proteins, which have an increased affinity for the glycolipids present on apical secretory vesicles (13, 14). In addition, the secretion of several other proteins has been proposed to be affected by their interaction with the membrane components of secretory vesicles (3, 8, 21, 39, 40). Because apoA-I and apoA-II were secreted relatively lipid-poor by MDCK cells (Fig. 5), there may be increased interaction of nascent apoA-I and apoA-II produced by MDCK cells for the membranes of the secretory pathway. Significant amounts of apical secretion may not have been observed in Caco-2 cells because, unlike MDCK cells, Caco-2 cells readily secrete apolipoproteins associated with lipid (4, 41, 42). The apical to basolateral ratios of apoA-I and apoA-II that we observed in Caco-2 cells were similar to the distribution of other proteins that are secreted by the basolateral default route (4, 5, 27, 42), which supports the default pathway secretion model in Caco-2 cells. It has been observed, however, that for mucosal explants of small intestines there is decreased apical secretion of apoA-I when there are excess levels of intracellular lipid (20). Similarly, the polarized apical secretion in MDCK cells of apoJ, which is primarily secreted basolaterally in hepatocytes, has been proposed to be due to the absence of lipoprotein formation in MDCK cells (21). Overall, these results suggest that although apoA-I and apoA-II may largely be secreted by a default route in both Caco-2 cells and MDCK cells, the ratio of apical to basolateral secretion may be modulated by the amount of intracellular lipid available for association to apolipoproteins during secretion.

In summary, this study suggests that the polarized secretion in MDCK cells of apoA-I and apoA-II is not mediated by a protein-dependent sorting signal but largely follows a cell-dependent default pathway. An additional mechanism has also been proposed to account for the apical bias observed in MDCK cells that involves the differential interaction of apolipoproteins with the secretory pathway. Future studies of the interaction of apolipoproteins with the membranes of the secretory pathway will be useful for further exploration of the mechanism of the polarized secretion of apolipoproteins. Finally, transfected MDCK cells should be, in general, a useful model for examining the biosynthesis and secretion of apolipoproteins in heterologous polarized cells.

We wish to thank J. Mallory for providing the pLen plasmid.

Manuscript received 11 February 1993 and in revised form 22 August 1994.

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