Only Multimeric Hensin Located in the Extracellular Matrix Can Induce Apical Endocytosis and Reverse the Polarity of Intercalated Cells

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When an intercalated epithelial cell line was seeded at low density and allowed to reach confluence, it located the anion exchanger band 3 in the apical membrane and an H+-ATPase in the basolateral membrane. The same clonal cells seeded at high density targeted these proteins to the reverse location. Furthermore, high density cells had vigorous apical endocytosis, and low density cells had none. The extracellular matrix of high density cells was capable of inducing apical endocytosis and relocation of band 3 to the basolateral membrane in low density cells. A 230-kDa extracellular matrix (ECM) protein termed hensin, when purified to near-homogeneity, was able to reverse the phenotype of the low density cells. Antibodies to hensin prevented this effect, indicating that hensin is necessary for conversion of polarity. We show here that hensin was synthesized by both low density and high density cells. Whereas both phenotypes secreted soluble hensin into their media, only high density cells localized it in their ECM. Analysis of soluble hensin by sucrose density gradients showed that low density cells secreted monomeric hensin, and high density cells secreted higher order multimers. When 35S-labeled monomeric hensin was added to high density cells, they induced its aggregation suggesting that the multimerization was catalyzed by surface events in the high density cells. Soluble monomeric or multimeric hensin did not induce apical endocytosis in low density cells, whereas the more polymerized hensin isolated from insoluble ECM readily induced it. These multimers could be disaggregated by sulfhydryl reagents and by dimethylmaleic anhydride, and treatment of high density ECM by these reagents prevented the induction of endocytosis. These results demonstrate that hensin, like several ECM proteins, needs to be precipitated in the ECM to be functional.

The plasma membrane of epithelial cells is polarized into two domains; apical and basolateral, each of which has characteristic protein and lipid composition (1–3). Although targeting sequences were found in the structure of several polarized proteins, recent studies have shown that some proteins are polarized in a cell type-specific manner; for instance the Na,K-ATPase is targeted to the apical membrane of retinal pigment epithelium but to the basolateral membrane of most other epithelia (4). Several other proteins exhibit this flexibility in targeting; the most dramatic example is the targeting of the proton-translocating ATPase and the Cl,HCO3 exchanger of the intercalated cell of the renal tubule (reviewed in Ref. 5). These cells exist in a spectrum of forms. One extreme, the α type, has an apical H+-ATPase and a basolateral anion exchanger that is an alternately spliced form of the erythroid band 3 (kAE1) and hence is capable of trans-epithelial secretion of H+. In contrast, the β form secretes HCO3 by a basolateral H+-ATPase and an apical kAE1 (6). However, one study did not find kAE1 by immunoblot analysis but confirmed the presence of its mRNA (7). In an immortalized clonal cell line (8), we demonstrated that β cells can be converted to an α form by changes in the seeding density (9) reproducing a previous demonstration in vivo induced by changes in the acid content of the diet (10). The development of the clonal cell line allowed us to begin to uncover the biochemical basis of this plasticity.

When the immortalized cells were seeded on filters at subconfluent density, they eventually formed epithelial monolayers capable of secreting HCO3 into the apical medium. These cells did not exhibit any apical endocytic activity; they had apical kAE1 and a basolateral H+-ATPase (9). Remarkably, when the same cells were seeded at confluent density, their phenotype was that of the α cells; i.e. they had vigorous apical endocytosis, apical H+-ATPase, and basolateral kAE1, changes that occurred within 24 h of seeding. Both phenotypes were stable in culture for as long as the cells were observed. Hence, it was not the assumption of cell to cell contact but rather the initial seeding density that rapidly induced a binary switch in phenotype. More recently, we discovered that low density cells were flat, and high density cells were columnar. Furthermore, the apical cytoskeleton was dramatically different in the two phenotypes; low density cells had sparse microvilli, no apical actin, villin, or cytokeratin 19, whereas high density cells had exuberant microvilli, abundant sub-apical actin, villin, and cytokeratin 19 (11). These studies show that the transition from β to α phenotype was remarkably similar to terminal differentiation of epithelia, especially as seen in the intestine during the transition from crypt to villus (12).

When low density cells were seeded on the extracellular matrix (ECM) of high density cells, they assumed all the characteristics of high density cells. By using the induction of apical endocytosis as an assay, we purified a protein from high density ECM that was capable of converting the polarity of the low density cells (9). This protein, now termed hensin (for change in polarity), was defrayed in part by the payment of page charges. This article must be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: kAE1, the kidney-specific alternately spliced form of the anion exchanger 1; DMMA, dimethylmaleic anhydride; DTT, dithiothreitol; ECM, extracellular matrix; NEM, N-ethylmaleimide; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; CUB, Complement subcomponents C1r/C1s, Uegf, Bmp; SRCR, scavenger receptor cysteine-rich; ZP, zona pellucida domain.
Precipitation of Hensin in ECM and Polarity Reversal

body in Japanese, see Ref. 13), is composed of three types of domains, SRCR (14), CUB (15), and Zp domains (16). Hensin is a protein widely expressed in epithelial organs and brain. During the purification of hensin, it became clear that the protein traveled on gel filtration at a mobility suggestive of a size much higher than that predicted by its molecular mass of 230 kDa. To purify hensin to homogeneity required treatment with SDS and 4 M urea (13), but this denatured, if pure, hensin did not induce apical endocytosis in low density cells. However, a polyclonal antibody generated against a fusion protein composed of two SRCR domains prevented the induction of endocytosis in high density cells (13). These results demonstrated that hensin is necessary for activity, but whether it is also sufficient by itself is at present unknown.

In the present paper, we demonstrate that hensin is secreted as a soluble protein into the basolateral medium by the two phenotypes. Low density cells secrete only monomeric hensin, whereas high density cells secrete a soluble form of hensin that is multimeric. In addition, high density cells (but not low density cells) secrete a form of hensin that is retained in the ECM as a higher order multimer. We refer to this insoluble ECM form of hensin as precipitated hensin pending further analysis of its structure. Many ECM proteins such as collagen and fibronectin are retained in the ECM because they form fibrils composed of a large number of monomers associated in a specific architectural pattern as fibers or networks. Procedures that disaggregate the ECM type of multimer prevent hensin from inducing apical endocytosis in low density cells. These results demonstrate that only multimeric insoluble hensin is capable of inducing endocytosis, reversing the polarity, and inducing terminal differentiation of intercalated cells.

MATERIALS AND METHODS

35S Labeling, Pulse-Chase, and Immunoprecipitation—Cloned C of 6-intercalated cells were maintained at 32 °C as described (8, 9). The cells were seeded on 0.45-μm Costar Corp., Cambridge, MA) at a density of 2 × 104 cells/cm2 (low density) or 4 × 104 cells/cm2 (high density) and cultured at 40 °C to inactivate T antigen. Cells were seeded on transwells and grown for 24 h at 40 °C. Media were changed to minimum Eagle’s medium without methionine and cysteine (Sigma) (0.5 mg/ml in 10 mM NaOH) was freshly prepared and added to the filters and incubated at 4 °C overnight before cells were seeded on low density cells. Sucrose Density Gradient Analysis of Hensin—Conditioned media were collected from low density or high density cells and concentrated 10-fold using Centricon-10 (Amicon, Beverly, MA) and divided into Eppendorf tubes. Each tube received 5 mM DTT or 5 mM EDTA. For the DMMA (5 mM) studies, the samples were incubated at 4 °C for an hour and then labeled with 50 μM Hepes-KOH (pH 8.5) for 5 min. These samples were further dialyzed against 50 μM Hepes-KOH (pH 6.7) and ultra centrifuged at 100,000 g for 16 h at 4 °C. Proteins in each fraction (1 ml) were migrated by 6% trichloroacetic acid, dissolved in a sample buffer, subjected to 7.5% SDS-PAGE followed by Western blotting with anti-hensin serum.

RESULTS

Hensin Is a Secretory Glycoprotein That Is Retained in the ECM of High Density Cells—Pulse-chase experiments showed that the molecular weight of hensin increased after its synthesis, and the intensity of the band eventually decreased with prolonged periods of chase (Fig. 1A). Hensin was secreted into the basolateral medium, partially explaining the reduced intensity during the chase period (Fig. 1C). The molecular weight shift was abolished when the cells were pretreated with tunicamycin indicating that hensin is N-glycosylated (Fig. 1B).

Surprisingly, hensin was synthesized not only in high density cells but also in low density cells; indeed low density cells consistently synthesized it at a higher rate (Fig. 2) (11). Cells were labeled with [35S]methionine, and hensin was chased into the cell lysate, ECM, and medium in both low density and high density cells. ECM (here defined as the guanidine-extractable material remaining on the filter after Triton solubilization.) While hensin accumulated in the ECM of high density cells, very little was seen in low density cells (Fig. 2). These results confirmed our original finding that high density ECM, but not that of low density, contained hensin (9, 13). Basolateral secretion was seen in both phenotypes, but the degree of polarization was not high since these cells were examined only 2 days after seeding, a time before the development of impermeable monolayers as assayed by the trans-epithelial flux of [14C]inulin (data not shown). Recent immunocytochemical...
studies documented that hensin is indeed an ECM protein in high density; it was accessible to externally added antibodies without the necessity for permeabilizing the cells, and it co-localized in confocal images with an authentic ECM protein, collagen IV (11). Fig. 3 shows that hensin began to accumulate in the ECM within 3 h after seeding the cells at high density. In low density cells, there was a large amount of hensin, but it was restricted to intracellular vesicles (Fig. 3).

Soluble Hensin Exists in Different Forms in the Two Phenotypes—Hensin was present in high density cells in both soluble (i.e. secreted into the media) and insoluble forms (i.e. present in the ECM). However, low density cells seemed to produce only the soluble form raising the possibility that hensin was degraded in ECM of low density but not that of high density cells. However, detailed experiments on the rate of proteolysis of hensin, the secretion of matrix metalloproteinases, and the effect of protease inhibitors on the half-life of hensin failed to reveal any difference between the two phenotypes (data not shown).

To examine whether even the soluble hensin might exist in different forms in the two phenotypes, we separated secreted hensin by sucrose density gradient centrifugation. We found that hensin secreted by low density cells was recovered in fractions 3 and 4, as would be predicted from monomeric hensin (Fig. 4). However, as much as 30% of hensin secreted into the media of high density cells was recovered in fractions 7 and 8 and in the pellet (Fig. 4). Fraction 7 corresponds to an apparent molecular mass of 700,000, double (or perhaps even quadruple) the apparent molecular weight of hensin. Because hensin is a cysteine-rich protein, we treated the high density media with dithiothreitol (DTT), a reagent that reduces disulfide bonds; all multimeric hensin was reduced to the monomeric form (Fig. 4, lane DTT). These results, however, cannot distinguish whether the multimerization was produced by inter-molecular or by intra-molecular disulfide bonds. Recent studies on the structure of SRCR domains demonstrated that intra-molecular disulfide bonds are critical for formation of the native structure of the domain (17).

DMMA is a useful reagent that reacts with the ε-amino group of lysine to produce a maleyl lysine, thereby converting it from a cation to an anion. This large change in electrostatic potential frequently results in disaggregation of subunits or large conformational changes (18). Maleyl lysine is stable at neutral or alkaline pH but is rapidly hydrolyzed in acidic media resulting in the regeneration of the cationic group of lysine. We used DMMA treatment to probe the association and dissociation of hensin. When the conditioned medium of high density cells was pretreated with DMMA at pH 8.0, hensin was substantially reduced in fractions 7 and 12 indicating that hensin unfolded to assume the monomeric form (Fig. 4, DMMA pH 8). These results demonstrate that hensin does not form multimers by inter-molecular disulfide bonds. When this sample was further dialyzed against a buffer of pH 6.7, 25% of the total amount of hensin appeared in fraction 7 demonstrating that when high density hensin is disassembled, it could spontane-
Conditioned media collected from low density (LD) or high density (HD) cells were treated with various reagents as shown at 4 °C for 1 h. The sample treated with DDMMA was dialyzed against 50 mM Hepes-KOH (pH 8.0) at 4 °C, and half of it was further dialyzed against 50 mM Hepes-KOH (pH 6.7) at 4 °C overnight. These samples were loaded on 5–30% sucrose gradient and ultracentrifuged at 100,000 g for 16 h at 4 °C. Proteins from each fraction were precipitated by 5% trichloroacetic acid, electrophoresed, and blotted with anti-hensin serum. Fraction 12 corresponds to the fraction were precipitated by 5% trichloroacetic acid, electrophoresed, and ultracentrifuged at 100,000 g for 16 h at 4 °C. Proteins from each fraction were precipitated by 5% trichloroacetic acid, electrophoresed, and blotted with anti-hensin serum. Fraction 12 corresponds to the bottom of the gradient. Aldolase (158 kDa), catalase (232 kDa), and thyroglobulin (669 kDa) were used as marker proteins.

Induction of apical endocytosis is an early and consistent marker for the change in phenotype; high density fraction suggesting that it is a multimer of much higher order than a tetramer. Soluble multimeric hensin of high density cells must undergo additional multimerization, fibril formation, or association with other ECM proteins before being able to localize in the ECM. Because divalent ions are often critical for stabilization of tertiary structure, we tested their effect on hensin extraction. High density ECM when treated with calcium (or other divalent ions) contained more hensin than those exposed to the chelators EDTA or EGTA (Fig. 5), suggesting that the localization of hensin in the ECM likely involves a calcium-mediated aggregation or a change in conformation. However, EDTA and EGTA did not affect the extent of multimerization needed to induce the change in phenotype, since the concentrated fractions did not induce apical endocytosis (Fig. 7A). To examine the extent of multimerization needed to induce the change in phenotype, we concentrated the soluble fractions 7 and 8 and fraction 12 and exposed low density cells to these fractions for 5 days and assayed for apical endocytosis. These concentrated fractions did not induce apical endocytosis (Fig. 7A) implying that such multimers were either not polymeric enough, of different conformation, or else required another protein to exert their effect. We point out that these multimers were still soluble since they were identified in the media of high density cells making it likely that only the polymeric form of hensin is functionally active. Pretreatment of high density ECM by DTT or N-ethylmaleimide or both sequentially to alkylate all reducible disulfide bonds (and therefore to convert hensin to a monomeric form) abolished the ability of high density ECM to induce apical endocytosis (Fig. 7B). When high density ECM-containing filters were pretreated with DTT or NEM, hensin was extracted to the same extent as from untreated filters. Hence, the lack of effect on endocytosis was not due to loss of hensin from these filters. Similarly, treatment

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**Fig. 4.** Separation by sucrose density gradients shows that soluble hensin exists in different forms. Conditioned media collected from low density (LD) or high density (HD) cells were treated with various reagents as shown at 4 °C for 1 h. The sample treated with DDMMA was dialyzed against 50 mM Hepes-KOH (pH 8.0) at 4 °C, and half of it was further dialyzed against 50 mM Hepes-KOH (pH 6.7) at 4 °C overnight. These samples were loaded on 5–30% sucrose gradient and ultracentrifuged at 100,000 g for 16 h at 4 °C. Proteins from each fraction were precipitated by 5% trichloroacetic acid, electrophoresed, and blotted with anti-hensin serum. Fraction 12 corresponds to the bottom of the gradient. Aldolase (158 kDa), catalase (232 kDa), and thyroglobulin (669 kDa) were used as marker proteins.

**Fig. 5.** Extraction condition affects binding of hensin in ECM. High density cells were labeled with 35S for 12 h. Cells were extracted first with Triton (Tx) with 1 mM calcium chloride or chelating agents, and the ECM deposits were subsequently extracted and immunoprecipitated.

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**Fig. 6.** Separation by sucrose density gradient of ECM hensin. High density (HD) cells were extracted with 1% Triton X-100 in the presence of 1 mM calcium chloride, and the ECM deposits were extracted with 1% deoxycholate, 50 mM Tris-HCl (pH 7.5) at room temperature followed by loading on 5–30% sucrose gradients and Western blotting. Half of the sample was treated with 5 mM DTT for 1 h at 4 °C before loading onto the gradient.

ECM Hensin Is a Higher Order Multimer—Soluble multimeric hensin of high density cells must undergo additional multimerization, fibril formation, or association with other ECM proteins before being able to localize in the ECM. Because divalent ions are often critical for stabilization of tertiary structure, we tested their effect on hensin extraction. High density ECM when treated with calcium (or other divalent ions) contained more hensin than those exposed to the chelators EDTA or EGTA (Fig. 5), suggesting that the localization of hensin in the ECM likely involves a calcium-mediated aggregation or a change in conformation. However, EDTA and EGTA did not change the multimeric state of soluble hensin secreted into the media (Fig. 4), suggesting that the effect of calcium in retaining hensin in the ECM might involve mechanisms other than multimerization; perhaps it had an effect on association with other ECM proteins.

When the ECM of high density cells was isolated (using deoxycholate extraction rather than guanidine to avoid excessive denaturation) and separated on sucrose density gradients, hensin remained in the pellet. Treatment of ECM by DTT resulted in a partial solubilization, where some hensin was now present in fractions as light as a tetramer, although the majority of hensin remained at a higher order (Fig. 6). These results demonstrate that ECM hensin precipitates in a very high density fraction suggesting that it is a multimer of much higher order than a tetramer.

Only Insoluble Polymeric ECM Hensin Is Capable of Inducing the Change in Phenotype—Induction of apical endocytosis is an early and consistent marker for the change in polarity; high density cells have a vigorous rate, whereas low density cells have no apical endocytosis (Fig. 7A). Low density cells seeded on high density matrix develop apical endocytosis (Fig. 7A). Incubation of high density cells in media containing a polyclonal antibody to hensin inhibited the development of apical endocytosis (Fig. 7A) (13). These results demonstrate that hensin in the ECM of high density cells is necessary for induction of apical endocytosis and, by extension, reversal of the polarity of several proteins in the low density cells.

What is the form of hensin that can induce the reversal of polarity? Soluble monomeric hensin, in fractions 3 and 4, was concentrated and applied to low density cells; it was unable to induce apical endocytosis in low density cells (Fig. 7A). To examine the extent of multimerization needed to induce the change in phenotype, we concentrated the soluble fractions 7 and 8 and fraction 12 and exposed low density cells to these fractions for 5 days and assayed for apical endocytosis. These concentrated fractions did not induce apical endocytosis (Fig. 7A) implying that such multimers were either not polymeric enough, of different conformation, or else required another protein to exert their effect. We point out that these multimers were still soluble since they were identified in the media of high density cells making it likely that only the polymeric form of hensin is functionally active. Pretreatment of high density ECM by DTT or N-ethylmaleimide or both sequentially to alkylate all reducible disulfide bonds (and therefore to convert hensin to a monomeric form) abolished the ability of high density ECM to induce apical endocytosis (Fig. 7B). When high density ECM-containing filters were pretreated with DTT or NEM, hensin was extracted to the same extent as from untreated filters. Hence, the lack of effect on endocytosis was not due to loss of hensin from these filters. Similarly, treatment
of high density ECM by DMMA also abolished its ability to induce apical endocytosis (Fig. 7B). These results demonstrate that only precipitated ECM hensin is capable of inducing the change in phenotype.

**High Density Cells Can Multimerize Extracellular Hensin**—The mechanism by which different forms of hensin exist in the two phenotypes could be due to a biosynthetic intracellular event, similar to what occurs during the initial stages of collagen biosynthesis (19). Alternatively, a cell-surface event in the high density phenotype occurs which will lead to polymerization and precipitation, a phenomenon described with fibronectin fibril formation. To distinguish between these possibilities, we labeled low density cells with $^{35}S$-methionine and concentrated the labeled hensin (i.e. fractions 3 and 4). This was then added to unlabeled low and high density cells. After a 24-h incubation, the media were removed, concentrated, and separated on sucrose density gradients. Hensin in all gradient fractions was immunoprecipitated and separated on SDS-PAGE, and $^{35}S$-labeled hensin was subjected to autoradiography and quantitated by densitometry. It is clear from Fig. 8 that high density cells were able to induce polymerization of hensin, whereas low density cells did not.

**DISCUSSION**

The functional assay used in this study, induction of apical endocytosis, is one of the cardinal events that occurs during the conversion of polarity in vitro and in vivo (9, 10). As an assay for this plasticity, it is more quantitative than the immunocytochemical localization of proteins, and the fact that low density cells had no apical endocytosis provides an excellent signal to noise ratio. High density cells (and $\alpha$ cells in situ) have vigorous apical endocytosis, and this process occurs early after seeding at high density and remains during the 2 weeks of observation. Since the vacuolar H$^+$-ATPase is packaged in sub-apical endocytic vesicles which continuously form and then fuse with the apical membrane, the induction of apical endocytosis is an excellent surrogate marker for the presence of the H$^+$-ATPase in the apical membrane (20–22). Furthermore, we recently found that high density cells have dramatically different apical cytoskeleton, such as the appearance of a sub-apical actin network and the induction of apical villin and cytokeratin 19 (11). These findings demonstrate that the induction of apical endocytosis, an important phenomenon in its own right, is also a legitimate marker for the reversal of polarity of the H$^+$-ATPase and the anion exchanger kAE1.

We initially found that the ECM of high density cells (defined as the 4 M guanidine extract after solubilizing cells with 1% Triton) contained an activity that retargeted kAE1 to the basolateral membrane and induced apical endocytosis in low density cells; the latter experiments were confirmed in the new studies shown in Fig. 7A. Purification using standard chromatographic methods resulted in a highly active faction whose major protein component was hensin (9). However, further purification of hensin required denaturation with 4 M urea and 0.1% SDS, a procedure that resulted in a homogeneously pure protein but that lacked the ability to induce apical endocytosis (13). Whether the loss of activity was due to denaturation of hensin, a loss of an associated protein, or whether hensin is unrelated to the polarity reversal required additional information. A fusion protein composed of two of the SRCR domains of hensin was used to generate polyclonal antibodies. These sera inhibited the ability of unfractionated high density ECM to induce apical endocytosis (results that are also recapitulated in Fig. 7A), whereas preimmune sera had no effect (13). Extensive morphological studies also show that anti-hensin antibodies...
also prevented the development of the above mentioned changes in apical cytoskeleton (11). Furthermore, seeding cells on purified ECM components (fibronectin, laminin, or collagen IV) or on complex ECM preparations (matrigel) did not induce the changes in apical cytoskeleton, nor did specific antibodies against ECM proteins prevent the change in phenotype (11). These results demonstrate that hensin is necessary for the reversal of polarity, but whether it is sufficient by itself to perform this activity or needs an additional protein will require more studies.

The surprising finding of the current study is that hensin was abundantly synthesized and secreted by low density cells, but it was not retained in the ECM. Because this soluble hensin appeared to be a monomer, it is likely that retention in the ECM requires oligomerization and or association with other ECM proteins. High density cells secreted soluble hensin into the medium and insoluble hensin that was localized in the ECM. The apparent molecular mass of the soluble hensin was as high as a tetramer. However, ECM hensin is much larger than a tetramer, and it precipitates in the ECM either alone or in association with other ECM proteins. Importantly, procedures that disrupted the multimeric formation inhibited the activity of hensin (Figs. 6 and 7). Furthermore, it appeared that multimerization to a lower order such as that which occurs in soluble hensin failed to induce the activity. Hence, these results demonstrate that only precipitated hensin can induce the change in phenotype.

What causes precipitation of hensin? We believe that studies on other ECM proteins might suggest an answer. Fibronectin and collagen are synthesized as soluble monomers, but their deposition in the ECM requires oligomerization and fibril formation, a process that is “catalyzed” by other proteins such as activated integrin receptors or proteases (19, 23, 24). The case of fibronectin is particularly instructive. Soluble fibronectin is unable to activate its receptor. However, “activation” of the receptor by a variety of procedures induces a high affinity state. This high affinity integrin receptor is capable of binding fibronectin and inducing it to form fibrils (23, 24). Can high density seeding perform the same function on the putative hensin receptor? Although this must await the identification of such a receptor, the studies of Fig. 8 demonstrate that addition of monomeric hensin produced by low density cells to cells seeded at high density causes hensin to form multimers, even precipitates. Of course, these studies do not exclude the role of other ECM proteins in helping to precipitate hensin. However, they demonstrate that oligomerization of hensin and its precipitation is not a biosynthetic event that occurs only in high density cells, similar to what occurs in collagen trimmer formation (19). Rather, the high density cells act from the outside to multimerize it.

We recently found that the change in phenotype induced by high seeding density includes the formation of exuberant microvilli, the localization of villin and cytokeratin 19 to the apical cytoplasm, and the assumption of a columnar shape by these epithelial cells (11). These findings are remarkably similar to what occurs in the intestinal epithelial cell when it differentiates from the crypt stem cell to a villus absorptive cell (12, 25). It has been demonstrated that some ECM component is involved in this terminal differentiation (26). Hensin is expressed in most epithelia, but its expression in the intestine is especially robust (13). We recently found that hensin is distributed in crypt cells in a pattern identical to that of low density intercalated cells, whereas in the villus cells its expression was like that of high density cells (13). These results suggest that hensin might be involved in terminal differentiation of other epithelia. Terminal differentiation is a critical step in epithelial biology whose interruption often leads to the development of malignancies. It was recently reported that chromosome 10q25-26 contains a region often deleted in malignant brain tumors, and a cDNA encoded by that region, DMBT1, was found to be deleted in 20% of malignant gliomas (27). We recently discovered that DMBT1 and hensin are alternately spliced forms of the same gene raising the possibility that hensin might be a tumor suppressor (28).

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REFERENCES

1. Eaton S., and Simons K. (1995) Cell 82, 5–8
2. Druzin, D. G., and Nelson, W. J. (1996) Cell 84, 335–344
3. Rodriguez-Boulan, E, and Powell, S. R. (1992) Annu. Rev. Cell Biol. 8, 395–427
4. Gundersons, D., Orlowksi, J., and Rodriguez-Boulan, E. (1991) J. Cell Biol. 112, 863–872
5. Al-Awqati, Q., Vijayakumar, S., Hikita, C., Chen, J., and Takito, J. (1998). Am. J. Physiol. 275, F183–F190
6. van Adelsberg, J. S., Edwards, J. C., and Al-Awqati, Q. (1993) J. Biol. Chem. 268, 11263–11269
7. Fejes-Toth, G, Chen, W. R., Rusvai, E., Moser, T., and Naray-Fejes-Toth, A. (1994) J. Biol. Chem. 269, 26717–26721
8. Edwards, J. C., van Adelsberg, J., Rater, M., Herzlinger, D., Lebowitz, J., and Al-Awqati, Q. (1992) Am. J. Physiol. 263, C521–C529
9. van Adelsberg, J., Edwards, J. C., Takito, J., Kiss, B., and Al-Awqati, Q. (1994) Cell 76, 1053–1061
10. Schwartz, G. J., Baraou, J., and Al-Awqati Q. (1985) Nature 318, 368–371
11. Vijayakumar, S., Takito, J., Hikita, C., and Al-Awqati, Q. (1999) J. Cell Biol. 144, 1057–1068
12. Louvard, D., Kedinger, M., and Hauri, H. P. (1992) Annu. Rev. Cell Biol. 8, 157–195
13. Takito, J., Hikita, C., and Al-Awqati, Q. (1996) J. Clin. Invest. 98, 2325–2331
14. Resnick, D., Pearson, A., and Krieger, M. (1994) Trends Biochem. Sci. 19, 5–8
15. Bork, P., and Beckmann, G. (1998) J. Mol. Biol. 231, 519–545
16. Bork, P., and Sander, C. (1992) FEBS LETT. 300, 237–240
17. Resnick, D., Chatterton, J. E., Schwartz, K., Slayter, H., and Krieger, M. (1990) J. Biol. Chem. 261, 26924–26930
18. Moses, G. K., and Peeny, R. E. (1971) Chemical Modification of Proteins. pp. 76–77, Holden-Day, San Francisco
19. Kadler, K. E., Holmes, D. F., Trotter, J. A., and Chapman, J. A. (1996) Biochim. J. 316, 1–11
20. Gluck, S., Cannon, C., and Al-Awqati, Q. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 4327–4331
21. Gluck, S., Kelly, S., and Al-Awqati, Q. (1982) J. Biol. Chem. 257, 9230–9233
22. Cannon, C., van Adelsberg, J., Kelly, S., and Al-Awqati, Q. (1985) Nature 314, 443–446
23. Wu, C., Keivens, V. M., O’Toole, T. E., McDonald, J. A., and Ginsberg, M. H. (1995) Cell 83, 715–724
24. Sechler, J. L., Takada, Y., and Schwarzbauer, J. E. (1996) J. Cell Biol. 134, 573–583
25. Simon, T. C., and Gordon, J. I. (1995) Curr. Opin. Genet. & Dev. 5, 577–586
26. Sandersn, I. R., Ezzell, R. M., Kedinger, M., Erlanger, M., Xu, Z. X., Pringault, E., Leon-Robine, S., Louvard, D., and Walker, W. A. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 7717–7722
27. Mollenhauer, J., Wiemann, S., Schurlein, W., Korn, B., Hayashi, Y., Wilgenbus, K. D., Deimling, A. V., and Pousta, A. (1997) Nat. Genet. 17, 32–39
28. Takito, J., Hikita, C., Vijayakumar, S., Warburton, D., and Al-Awqati, Q. (1999) Am. J. Physiol., in press