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Tubulogenesis: MDCK Cell Polarity Is Transiently Rearranged without Loss of Cell–Cell Contact during Scatter Factor/Hepatocyte Growth Factor-Induced Tubulogenesis

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Many organ systems are composed of networks of epithelial tubes. Recently, molecules that induce development of epithelial tubules and regulate sites of branching have been identified. However, little is known about the mechanisms regulating cell rearrangements that are necessary for tubule formation. In this study we have used a scatter factor/hepatocyte growth factor-induced model system of MDCK epithelial cell tubulogenesis to analyze the mechanisms of cell rearrangement during tubule development. We examined the dynamics of cell polarity and cell–cell junctions during tubule formation and present evidence for a multistep model of tubulogenesis in which cells rearrange without loss of cell–cell contacts and tubule lumens form *de novo.* A three-dimensional analysis of markers for apical and basolateral membrane subdomains shows that epithelial cell polarity is transiently lost and subsequently regained during tubulogenesis. Furthermore, components of cell–cell junctional complexes undergo profound rearrangements: E-cadherin is randomly distributed around the cell surface, desmoplakins I/II accumulate intracellularly, and the tight junction protein ZO-1 remains localized at sites of cell–cell contact. This suggests that differential regulation of cell–cell junctions is important for the formation of tubules. Therefore, during tubulogenesis, cell–cell adhesive contacts are differentially regulated while the polarity and specialization of plasma membrane subdomains reorganize, enabling cells to remain in contact as they rearrange into new structures. **© 1998 Academic Press**

Key Words: **tubulogenesis; epithelial polarity; cell–cell adhesion; kidney; morphogenesis.**

INTRODUCTION

Tubulogenesis is a developmental process common to the formation of many organs, including lung, salivary gland, mammary gland, pancreas, and kidney. Recent studies have shown that induction of morphogenesis of epithelial tubules in several different organs requires a complex interplay of many factors and receptors (Kjelsberg *et al.,* 1997; Sakurai *et al.,* 1997a,b; Sariola and Sainio, 1997; Vainio and Muller, 1997). For instance, during mammalian metanephric kidney development, glial cell-derived neurotrophic factor and its receptors, c-ret and GFRα-1, have been shown to be involved in induction of tubulogenesis from the ureteric bud (Moore *et al.,* 1996; Pichel *et al.,* 1996; Sanchez *et al.,* 1996; Schuchardt *et al.,* 1994). Similarly, genetic analysis has identified several genes that control initiation of branching and direction of outgrowth of epithelial tubules during tracheal development in *Drosophila* (Klambt *et al.,* 1992; Sutherland *et al.,* 1996). In addition, antibody treatment of cultured kidney organ rudiments has demonstrated that scatter factor/hepatocyte growth factor (SF/

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HGF) can regulate tubule development (Woolf *et al.,* 1995). These growth factors and receptors can be considered the "upstream" regulators of tubulogenesis.

The "downstream" response to induction of epithelial tubulogenesis presumably involves cell movements and changes in cell adhesion, intercellular junctions, and cell polarity. In contrast to the inductive signals, downstream responses during epithelial tubulogenesis have previously received relatively little discussion in the cell biological literature (Gumbiner, 1992), perhaps because of the relative difficulty of examining tubulogenesis in experimental systems that have often involved the culturing of embryonic organ rudiments. In this paper we studied the downstream responses to induction of tubule formation in order to understand the mechanisms of cell rearrangement during tubulogenesis.

A significant advance for studying mechanisms of tubulogenesis was made by the development of an *in vitro* tubulogenesis model system using Madin–Darby canine kidney (MDCK) epithelial cells induced by SF/HGF (Montesano *et al.,* 1991a,b). The bipartite name of SF/HGF reflects that it was independently isolated for two separate functions. SF was first identified as a factor in fibroblast-conditioned medium that, when added to MDCK cells grown as small islands on an impermeable support, causes the cells to scatter. HGF was defined by its ability to induce mitogenesis of cultured hepatocytes. It was later discovered that these two factors are identical and that SF/HGF is a ligand for the c-met protooncogene (c-met), a receptor tyrosine kinase (Birchmeier and Birchmeier, 1993; Comoglio, 1993; Furlong, 1992; Rosen *et al.,* 1994). Previous studies have shown that SF/HGF induces collagen gel cultures of various epithelial cell types to undergo morphogenesis into organ-like structures that have characteristics of the organ from which the cells were derived (Brinkmann *et al.,* 1995). MDCK cells form hollow fluid-filled cysts when cultured in collagen gels. Exposure of these preformed epithelial cysts to SF/HGF causes the cysts to develop branching tubules in a process that resembles tubulogenesis *in vivo.*

When grown as a monolayer on a permeable filter support, MDCK cells form a well-polarized epithelial monolayer, exhibiting apical and basolateral plasma membrane domains with unique compositions and well-defined cell–cell junctional complexes containing tight junctions (TJ), adherens junctions, and desmosomes (DS) (Drubin and Nelson, 1996; Rodriguez-Boulan and Nelson, 1989; Weimbs *et al.,* 1997). Previous studies have shown that MDCK epithelial cells within both initial cysts and end-stage lumen-containing tubules are polarized with microvilli on lumenal membranes, distinct apical and basolateral membranes, and cell–cell junctional complexes on lateral membrane borders (Montesano *et al.,* 1991b; Santos *et al.,* 1993; Wang *et al.,* 1990a). However, the dynamic changes in localization of apical/basolateral membrane markers and cell–cell junctional proteins during the transition between cyst and tubule have not been identified.

SF/HGF-induced tubulogenesis of MDCK cells in culture provides an excellent model system to study MDCK cell polarity and cell–cell interactions during tubulogenesis. Studies in which this system has been manipulated by genetic or pharmacological means have already proven to be a rich source of information on the mechanisms of tubulogenesis (Barros *et al.,* 1995; Boccaccio *et al.,* 1998; Cantley *et al.,* 1994; Crepaldi *et al.,* 1997; Derman *et al.,* 1995; Dugina *et al.,* 1995; Sachs *et al.,* 1996; Sakurai *et al.,* 1997a; Sakurai and Nigam, 1997; Santos *et al.,* 1993; Santos and Nigam, 1993; Weidner *et al.,* 1995). For instance, we have previously used this system to express a mutant β-catenin and show that correct interaction of β-catenin with APC protein is essential for a very early step in tubulogenesis (Pollack *et al.,* 1997). Similar studies have shown that $\alpha2$ integrins are critical for tubule formation (Berdichevsky *et al.,* 1994; Saelman *et al.,* 1995; Schwimmer and Ojakian, 1995). Moreover, recent work has dissected signaling pathways regulating tubulogenesis that are activated through the c-met receptor (Boccaccio *et al.,* 1998; Ponzetto *et al.,* 1994; Royal *et al.,* 1997; Sachs *et al.,* 1996; Weidner *et al.,* 1996, 1995). Given the pace of past progress and the future potential using this system, it is imperative to define the basic nature of the cell rearrangements and changes in cell adhesion, junctions, and polarity during tubule formation. This analysis will form the basis for the dissection of the mechanisms of cell rearrangements during tubulogenesis.

Several different models have been suggested to explain the morphogenetic mechanisms by which cells rearrange to form tubules. A two-stage dissociation/reassociation model proposed by Thiery and Boyer (1992) attempted to reconcile the apparently contradictory actions of SF/HGF in inducing scattering of MDCK cells grown on plastic versus tubulation of MDCK cells grown in collagen gels. In the first stage of this model, MDCK cells would detach from the cyst, lose their polarity, and migrate as single cells out from the cyst and into the surrounding collagen gel. In the second stage, the migrating single cells would coalesce and reassemble into multicellular structures that form tubules of polarized cells. A contrasting model, implicit in much of the discussions of tubulogenesis, envisions that tubules form as "outpouchings" from the cyst; in this model the lumen of the developing tubule is always continuous and directly connected to the lumen of the cyst (Gilbert, 1994; Sariola and Sainio, 1997). These two models make very different predictions not just about cell rearrangements, but about cell–cell adhesion, cell junctions, and cell polarity. In the two-stage dissociation/reassociation model, cells transiently lose all cell–cell adhesion and probably most, if not all, polarity, before reestablishing these *de novo.* In contrast, the outpouching model predicts that the cells never lose adhesion or polarity and remain in a monolayer organized around a lumen.

To test the mechanisms of cell rearrangement in tubulogenesis we investigated epithelial cell–cell interactions and polarity during transitional stages in the development of lumen-containing tubules by examining the spatiotemporal

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TABLE 1
Protein Markers for Epithelial Cell Polarity and Cell–Cell Junctions

Protein	Location in polarized epithelial cells	Proposed function	References
E-cadherin	Basolateral plasma membrane	Adherens junction cell–cell adhesion	Takeichi, 1991
gp135	Apical plasma membrane	?	Ojakian and Schwimmer, 1988
Desmoplakins I and I	Cytoplasmic plaque components of desmosomes on lateral plasma membrane	Desmosome cell–cell adhesion	Pasdar *et al.*, 1988b Garrod, 1993
ZO-1	Peripheral membrane component of tight junctions at the border between apical and basolateral plasma membrane	Tight junction cell–cell adhesion	Stevenson *et al.*, 1986

distribution of markers for apical and basolateral membranes and cell–cell junctions at multiple time points throughout tubule formation. We find that during the transition from a cyst to a tubule there is an initial loss of cell polarity, which is reestablished as lumen formation proceeds. We also find that lumen formation occurs *de novo* at sites of cell–cell contact, rather than by extrusion of the cyst lumen. Cell–cell adhesion is maintained throughout tubulogenesis. However, the patterns of relocalization of components of adherens junctions, tight junctions, and desmosomes are distinct and suggest that differential regulation, rather than disruption of cell–cell associations, is an important mechanism for tubulogenesis.

MATERIALS AND METHODS

Cell Culture

MDCK type II cells (originally isolated at the EMBL in Heidelberg) (Louvard, 1980) were maintained in MEM containing Earle's balanced salt solution (MEM-EBSS; Cellgro, Mediatech, Inc., Washington, DC) supplemented with 5% FCS (Hyclone, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2/95% air. For growth of cells in three-dimensional collagen gels, MDCK cells were trypsinized and then triturated at room temperature into a single-cell suspension of 5×10^4 cells/ml in a type I collagen solution containing 66% Vitrogen 100 (3 mg/ml; Celtrix, Palo Alto, CA), 1× MEM, 2.35 mg/ml $NaHCO_3$, 0.02 M Hepes, pH 7.6, and 12% dH_2O. Cells in suspension were then plated onto Nunc filters (Cat. No. 162243, 10 mm, 0.02- to 0.2-μm pore size; Applied Scientific, San Francisco, CA). The type I collagen solution was allowed to gel by incubation at 37°C, 95% air/5% CO_2 prior to addition of medium. Cultures were fed every 3–4 days and grown for 10–12 days, until MDCK cell cysts with lumen were formed.

Antibodies/Reagents

Hybridoma cells secreting mouse anti-E-cadherin mAb (rr1; Gumbiner and Simons, 1986) were a kind gift from B. Gumbiner (Sloan–Kettering, New York, NY). Rat mAb R40.76 against ZO-1, a tight junction peripheral membrane protein (Anderson *et al.*, 1988; Stevenson *et al.*, 1986), was obtained from B. Stevenson or Chemicon (Chemicon International, Temecula, CA). Rabbit polyclonal Ab against bovine desmosomal proteins desmoplakins I and II (dpI/II; Pasdar and Nelson, 1988a) was generously provided by M. Pasdar (University of Alberta, Edmonton, Canada) and W. J. Nelson (Stanford University, Stanford, CA). Mouse mAb 3F2 supernatant against gp135, a MDCK apical membrane glycoprotein (Ojakian and Schwimmer, 1988), was kindly provided by W. J. Nelson (Stanford University) and G. Ojakian (SUNY Health Science Center, Brooklyn, NY). The marker proteins identified by the above antibodies are described in Table 1. All secondary antibodies used for immunofluorescence (goat anti-mouse–FITC, goat anti-rat–FITC, and goat anti-rabbit–FITC) were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). Propidium iodide (ppI) was purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant human HGF was generously provided by R. Schwall (Genentech, South San Francisco. CA).

Production of Fibroblast-Conditioned Media Containing SF/HGF

To obtain conditioned medium containing SF/HGF, MRC5 human lung fibroblasts (ATCC CCL171) were grown to confluence in DME (DMEH21, obtained from the UCSF cell culture facility) containing 10% FBS (Hyclone, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin in T75 tissue culture flasks (Corning, Corning, NY) or, for 2× conditioned medium, in T150 ridged tissue culture flasks (Corning). The medium was replaced with 30 ml of fresh medium and cell culture was continued for 3 days. Conditioned medium was collected, centrifuged to remove cell debris, and frozen at −20°C until use. Control conditioned medium was obtained by growing D550 human foreskin fibroblasts (kindly provided by Ward D. Peterson, Detroit Medical Center, Detroit, MI) under the same culture conditions as for MRC5 cells, but in MEM-EBSS supplemented with 0.1 mM nonessential amino acids ((100× stock, UCSF cell culture facility), 0.1% lactalbumin hydrolysate (Sigma), 0.1 mg/ml sodium pyruvate (100× stock, UCSF cell culture facility), and 10% FBS.

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Treatment of MDCK Cysts with SF/HGF-Containing Media

Three-dimensional cultures of MDCK cells grown to form cysts were treated for various numbers of days with D550 or MRC5 fibroblast-conditioned media diluted 1:1 with MEM-EBSS, 5% FBS. Cultures were refed daily with freshly diluted conditioned medium.

Immunofluorescence and Confocal Microscopy

Cells in collagen gel cultures were processed for immunofluorescence at room temperature as follows: cultures were rinsed with PBS, pH 7.4, containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBS^+), fixed for 30 min with 4% paraformaldehyde in PBS^+, permeabilized for 30 min with 0.025% saponin in PBS^+, rinsed with PBS^+, and quenched for 10 min with 75 mM NH_4Cl, 20 mM glycine in PBS^+, pH 8.0. Nonspecific binding sites were blocked by rocking for 30 min in PBS^+, 0.025% saponin, 0.7% fish skin gelatin (block buffer) followed by 10 min in block buffer + 0.1 mg/ml boiled RNase A. Samples were then rocked 1–3 days at 4°C in primary antibodies diluted into blocking buffer containing 0.02% azide. Primary antibody concentrations were as follows: rr1 mAb supernatant, 3:1; R40.76 anti ZO-1 ascites, 1:150; R40.76 anti ZO-1 mAb supernatant, 3:1; rabbit anti-dpI/II, 1:100; and gp135 mAb supernatant, 3:1. After extensive washing with PBS^+/saponin and blocking buffer, samples were incubated overnight at 4°C in blocking buffer containing 0.02% azide with FITC-conjugated secondary antibodies diluted 1:100 and ppI diluted 1:1000 from a 3–4 mg/ml stock. Samples were then washed extensively, postfixed with 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4, and mounted in Vectashield (Vector Labs, Burlingame, CA). Specific staining was clearly detected in confocal images of collagen gel samples. However trapping of secondary antibody aggregates within the collagen gel sometimes resulted in bright spots of staining in the collagen gel surrounding the cell cultures.

Confocal images were collected using a krypton–argon laser with K1 and K2 filter sets coupled to a Bio-Rad MRC600 confocal head and an Optiphot II Nikon microscope with a Plan Apo 60× 1.4 NA objective. Collagen gel cultures were imaged in the *x–y* plane of the sample with a motor step size of 0.5 or 1 μm, Kalman filtering with five frames/image, and the diaphragm set at 1/3 open. To ensure a complete view of the complex three-dimensional aspects of the structures that were studied, we always obtained sets of serial sections collected in the *x–y* plane that completely spanned through control cysts or tubule-containing structures. Generally the figures shown present either selected individual *x–y* sections or projections of several consecutive sections that were selected from these complete serial sections. The data were analyzed using Comos and NIH Image software. Images were converted to TIFF format. Contrast levels of the images were adjusted and composites were prepared using Adobe Photoshop (Adobe Co., Mountain View, CA) on a PowerMacIntosh 7200/75 (Apple, Cupertino, CA).

RESULTS

Spatial and temporal changes in epithelial cell surface domains were analyzed during tubulogenesis by examining the distribution of marker proteins for apical, basolateral, and junctional membrane specializations during the development of tubules from SF/HGF-stimulated MDCK cell cysts grown in collagen.

Development of Lumen-Containing Tubules from MDCK Cell Cysts Occurs in Four Distinct Stages

To investigate how cell–cell interactions and polarity are altered during morphogenetic transitions in the development of lumen-containing tubules from polarized cysts, we studied MDCK cell cysts stimulated for various amounts of time with medium containing SF/HGF, a factor known to induce epithelial tubulogenesis.

MDCK cell cysts were treated between 0 and 7 days with either a control conditioned medium that does not contain SF/HGF or a SF/HGF-containing conditioned medium. Since other growth factors that act as upstream inducers of tubulogenesis may be present in the conditioned medium, we also examined cultures stimulated with purified recombinant human SF/HGF. Similar results were obtained with purified recombinant human SF/HGF (data not shown), suggesting that the downstream events examined in this study were induced by either conditioned medium or purified SF/HGF. Cell morphology and polarity were analyzed in individual confocal sections and projections of serial confocal sections by immunofluorescence detection of nuclei (ppI staining) and cell membrane proteins E-cadherin and GP135. Morphological transitions that were observed during tubule development are first shown diagrammatically in Fig. 1 and are supported in data presented below. Although morphogenesis of lumen-containing tubules occurs as a continuous process, we observe four distinct stages of tubule development which we define as follows: (1) extensions, (2) chains, (3) cords, and (4) tubules. Cysts (Fig. 1A) are composed of a spherical monolayer of MDCK cells that seals off a fluid-filled central lumen. Extensions, formed after 1 day of stimulation, consist of membrane protrusions from individual cells of the cyst that extend into the extracellular matrix (Fig. 1B). Chains of cells that are connected to the cyst develop within 1–3 days of stimulation (Fig. 1C). After 3–5 days of SF/HGF treatment, cords form that are two to three cells thick and develop discontinuous lumens (Fig. 1D). Finally, tubules develop in which discontinuous lumens have enlarged, coalesced, and become continuous with the lumen of the cyst (Fig. 1E). The development of tubules in this system is sequential, although not completely synchronous, and tubules in several stages of development may be found emanating from a single cyst. Therefore, we present our data in terms of morphological stages rather than absolute time after addition of SF/HGF-containing medium.

Extension and Chain Stages of Tubulogenesis: Polarity of MDCK Membrane Domains Is Lost in Cells That Extend from a Cyst to Form a Branching Chain

To analyze epithelial membrane polarity during SF/HGF-induced tubulogenesis we collected stacks of 1-μm serial

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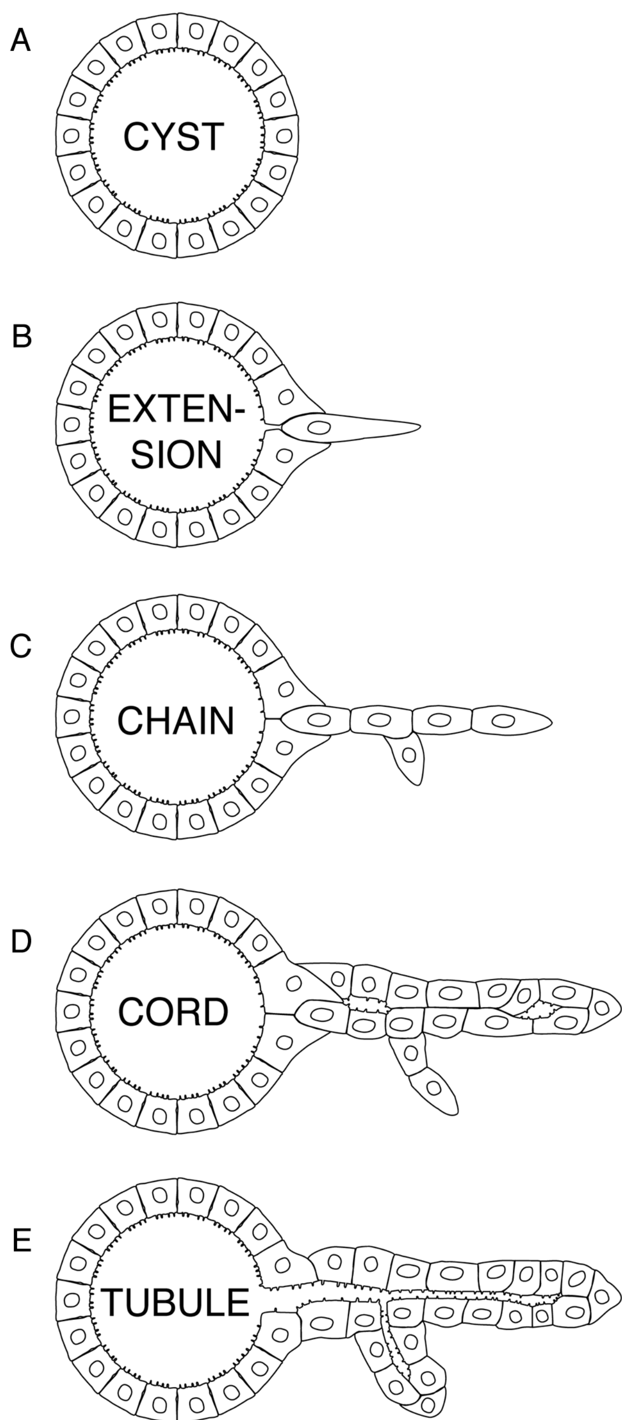

FIG. 1. Model of tubule morphogenesis. Development of tubules from polarized cysts is a continuous process that passes through four morphologically distinguishable stages which we have defined as follows: (A, stage 0) polarized cysts, (B, stage 1) extensions, (C, stage 2) chains, (D, stage 3) cords, and (E, stage 4) lumen-containing tubules. Microvilli are indicated as small membrane projections into the lumens. See text for detailed explanation.

confocal sections of immunofluorescently labeled samples from various stages of tubule development. We analyzed many single images and projections of image stacks for the localization of E-cadherin and GP135, well-known markers for basolateral and apical membranes, respectively (Behrens *et al.,* 1985; Gumbiner and Simons, 1986; Gumbiner *et al.,* 1988; Le Bivic *et al.,* 1990; Ojakian and Schwimmer, 1988; Shore and Nelson, 1991; Vestweber and Kemler, 1985). E-cadherin is a cell–cell adhesion protein that concentrates in the region of the apical junctional complex of MDCK cells (Nathke *et al.,* 1994), but is expressed throughout the basolateral membrane and maintains cell–cell contacts through calcium-dependent homotypic interactions with adjacent cells (Takeichi, 1991). Intracellular targeting assays have shown that in this strain of MDCK II cells 98% of newly synthesized E-cadherin is targeted directly to the basolateral surface after synthesis (Crepaldi *et al.,* 1994). The function of gp135 has not been identified. Samples were double-labeled with ppI to denote the localization of nuclei relative to these membrane markers. Representative individual confocal sections, with images split to distinguish membrane markers from nuclear staining, are shown in Fig. 2.

MDCK cell cysts grown in the absence of SF/HGF stimulation are shown in Figs. 2A and 2B. In cysts, E-cadherin is localized predominantly at lateral membranes in regions of cell–cell contact but is also found at membranes that are in contact with the extracellular matrix (Fig. 2A, arrows). E-cadherin is absent at membranes facing the lumen of the cyst (Fig. 2A, filled arrowheads). Gp135 is localized toward the interior of the cyst relative to ppI-stained nuclei and delineates the lumenal membrane of MDCK cell cysts (Figs. 2B and 2B′, arrows). Some gp135 appears to be within the cyst lumen, but most likely represents staining of apical microvilli. Gp135 is clearly absent from lateral cell–cell borders and cell–substrate contacts in MDCK cell cysts. These results confirm that MDCK cell cysts grown in three-dimensional collagen gel cultures are polarized with markers of the basolateral surface at cell–cell and cell–ECM borders and markers of the apical surface at lumenal membranes of the cyst.

The first stage of SF/HGF-induced tubule formation, development of extensions, is shown in Figs. 2C and 2D. During extension formation, individual cells of a cyst are stimulated to protrude into the surrounding collagen matrix. E-cadherin staining surrounds the cellular extensions, localizing at both cell–substrate and cell–cell borders (Fig. 2C, arrows). The open arrowhead in Fig. 2C points out the mouth of a narrow lumenal space between two cells that adjoin opposite sides and project under the extending cell where it has receded from the cyst. The filled arrowhead in Fig. 2C points to the remaining lumenal surface of the extending cell. Note that this surface is devoid of E-cadherin. E-cadherin remains at lateral membrane borders of cells in the cyst wall that are not extending. Note, however, that often cysts are not perfect hollow spheres (e.g., in Fig. 2C) and E-cadherin is also found at lateral

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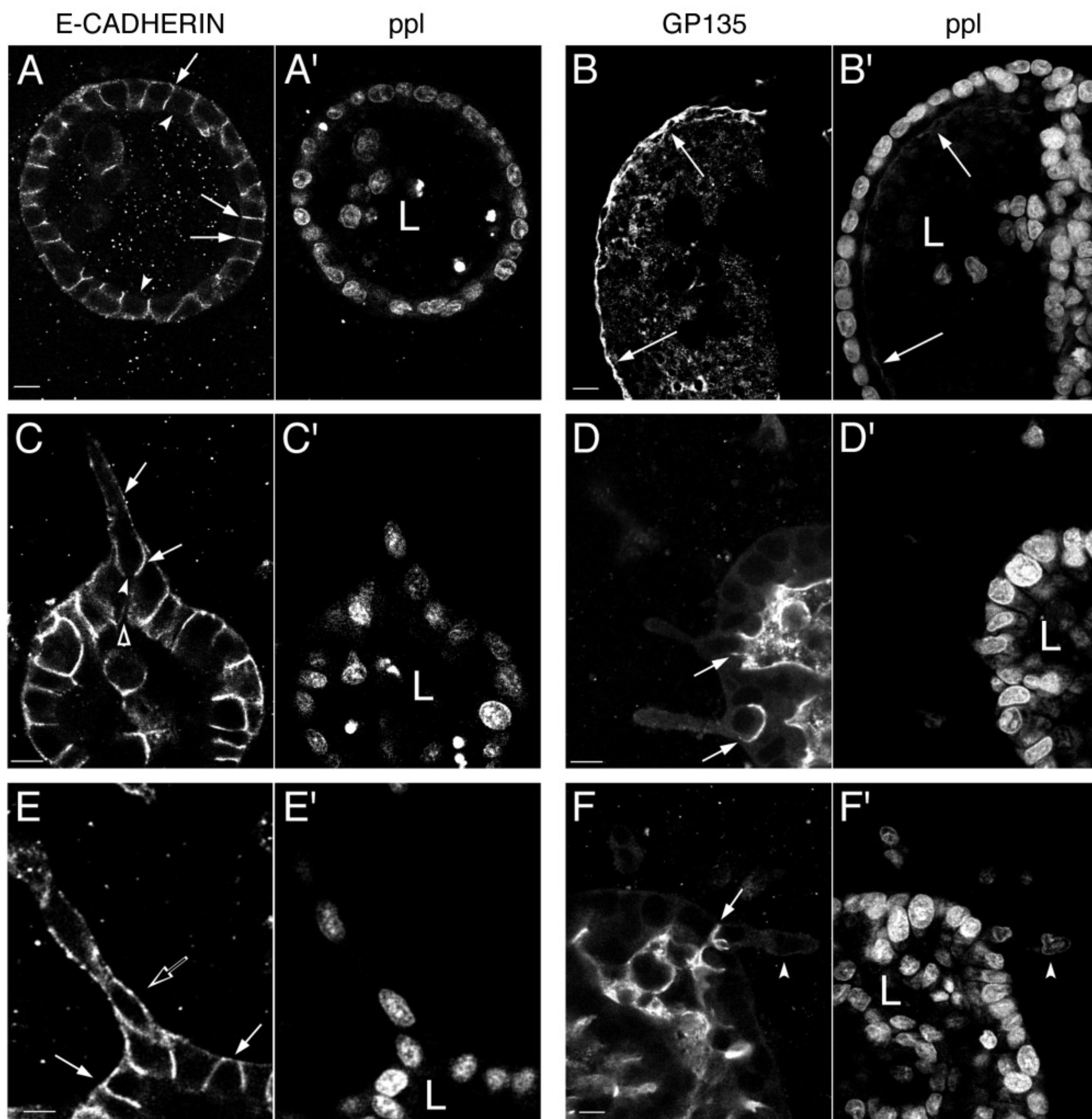

FIG. 2. Distinct apical/basolateral polarity is lost during early stages of tubulogenesis. In A–F′ each image pair represents a single confocal section taken from serially sectioned samples that were scanned simultaneously for FITC and ppI. Images are split so that staining for E-cadherin (A, C, and E) or gp135 (B, D, and F) is in the left half and nuclear staining (ppI; A′–F′) is in the right half. E-cadherin is strictly basolateral in polarized cysts (A, closed arrows), as E-cadherin staining is not detected at apical lumenal membranes (A, closed arrowheads). Closed arrows in B and B′ are aligned to demonstrate that gp135 is strictly localized to the apical membrane facing the lumen of polarized cysts. During the formation of cellular extensions (C, C′) E-cadherin staining is observed on membranes surrounding the extension in regions of cell–cell and cell–substrate contact (closed arrows). E-cadherin is excluded from the lumenal membrane (closed arrowhead), indicating that apical/basolateral polarity is retained. Cells neighboring the site of tubule initiation project apically under the extending cell (open arrowhead). Gp135 staining in D (closed arrows) indicates that polarity of apical membranes is retained in extensions. Cells in chains are completely surrounded by E-cadherin (E, open arrow) but basolateral polarity of E-cadherin is maintained in the cyst (E, closed arrows). Gp135 staining has largely disappeared from cells in chains and only background staining remains (F and F′, closed arrowheads). At the lumenal surfaces of cells within the cyst and at the base of the chain gp135 staining is retained (F, closed arrow). L, lumen. Bar, 10 μm.

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cell–cell borders between cells of the cyst wall and cells within the cyst. Gp135 is localized at the remaining lumenal membrane of the extending cells, establishing that at this stage of tubule development distinct apical membranes are maintained (Fig. 2D, arrows). Note that cells remain attached to the cyst during migration into the ECM to form extensions (Figs. 2C and 2D). These results demonstrate that during initial stages of tubulogenesis, membrane morphology is altered but apical and basolateral subdomains remain distinct.

The second stage of tubulogenesis, chain formation, is shown in Figs. 2E and 2F. In this stage, single-file chains of cells that are either linear (Figs. 2E, 2E′) or branching (data not shown) develop. E-cadherin is localized at both cell–cell and cell–ECM contacts, circumscribing each cell of the chain (Fig. 2E, open arrow). Thus, E-cadherin is randomly distributed at membrane surfaces of cells in the chain. In contrast, basolateral polarity of E-cadherin is maintained in cells of the cyst wall that are not undergoing morphogenesis (Fig. 2E, closed arrows). Gp135 staining disappears from cells in a short chain (Fig. 2F, arrowheads), indicating that apical membrane polarity is lost. In contrast, gp135 staining is retained at the lumenal surfaces in cells within the cyst, including cells at the base of the chain (Fig. 2F, arrow). Occasionally, intensely stained intracellular patches of GP135 are also observed (data not shown), suggesting that some of the gp135 may also be associated with intracellular vesicles (Vega-Salas *et al.,* 1988). These results show that MDCK cells lose apical/basolateral polarity but retain cell–cell contacts during reorganization into chains.

Cord and Tubule Stages of Tubulogenesis: MDCK Cell Polarity Is Regained as a Tubule Lumen is Formed

Subsequent stages of tubulogenesis were analyzed in serial confocal sections and three-dimensional image projections. Representative single confocal sections of cords and tubules, double-stained for ppI and either E-cadherin or gp135, are shown in Fig. 3. Figure 3A shows a cord two to three cells in diameter that is devoid of a lumen. E-cadherin surrounds each cell in the cord, localizing to regions of cell–cell and cell–substrate contact. These results were confirmed by careful analysis of serial sections and projections (data not shown). Gp135 staining is detected at or near cell–cell borders formed during the development of cords (Fig. 3B, arrows) and is not found on membranes in regions of cell–substrate contact (Fig. 3B, arrowhead). Sites of cell–cell contact at which gp135 is localized may be described as "protolumens," since they may indicate the location of future lumen formation. It is also possible that minute lumens that are below the resolution of the confocal microscope exist in these regions. These results show that the localization of the apical membrane marker, gp135, is highly polarized in regions of cell–cell contact upon formation of cords. In contrast, the basolateral membrane marker, E-cadherin, is found over the entire cell surface and overlaps regions of gp135 localization at cell–cell contacts in cords.

The maturation of cords into tubules is shown in Figs. 3C and 3D. Discontinuous lumens (Fig. 3C′, L) develop at sites of cell–cell contact. E-cadherin completely surrounds each cell in regions that retain cord-like structure (Fig. 3C, open arrow). In contrast, the basolateral polarity of E-cadherin is restored in lumen-containing regions of developing tubules (Fig. 3C, closed arrows). As tubule maturation proceeds lumens enlarge and become continuous with the lumen of the cyst (Fig. 3D′, L). Gp135 is localized at "free" cell surfaces facing morphologically distinguishable tubule lumens (Fig. 3D, arrows). We conclude from these results that tubules develop via *de novo* formation of lumens within cords at discrete sites of cell–cell contact. In addition, these results suggest that apical/basolateral membrane polarity is restored during *de novo* lumen formation.

SF/HGF-Induced Tubulogenesis Causes Differential Rearrangement of Desmosomal, Tight Junction, and Adherens Cell–Cell Junction Proteins

We have shown above that cells remain in contact as they rearrange to form tubules. However, E-cadherin localization is dramatically altered during sequential stages of tubulogenesis. Randomization of E-cadherin distribution during the chain and cord stages implies that cell–cell adhesion is modified during SF/HGF-induced tubulogenesis. To further understand the mechanisms through which cell–cell contacts are maintained as cells rearrange during tubulogenesis, we analyzed the localization of tight junction and desmosomal proteins at various stages of tubulogenesis.

In polarized epithelia, multiple DS are located along lateral membranes and function to maintain lateral cell–cell contacts (Garrod, 1993). To establish the localization of DS during tubulogenesis samples were labeled with an antibody that recognizes both desmoplakins I and II (dpI/II), cytoplasmic plaque components of the desmosomal complex (Pasdar *et al.,* 1991; Pasdar and Nelson, 1988b). Nuclei were counterstained with ppI. Serial confocal sections of many cyst cultures treated for various amounts of time with either control or SF/HGF-containing medium were collected and analyzed. Representative individual confocal sections are shown in Fig. 4. The distribution of dpI/II in polarized MDCK cell cysts is shown in Fig. 4A. Note that this confocal image captures cells of the cyst both in cross section (Figs. 4A and 4A′, lower left) and as a grazing section across the curvature of the cyst (Figs. 4A and 4A′, upper right). DpI/II appeared in punctate spots along basolateral plasma membranes of cells in polarized MDCK cell cysts (Fig. 4A, arrowheads). After stimulation by SF/HGF, dpI/II accumulated in large intracellular pools within extensions of cells that responded to SF/HGF by protruding into the extracellular matrix (Figs. 4B and 4B′, aligned arrows). However, dpI/II were also localized in punctate clusters that are likely to be desmosomal plaques at cell–cell borders between the extending cell and cells of the cyst wall

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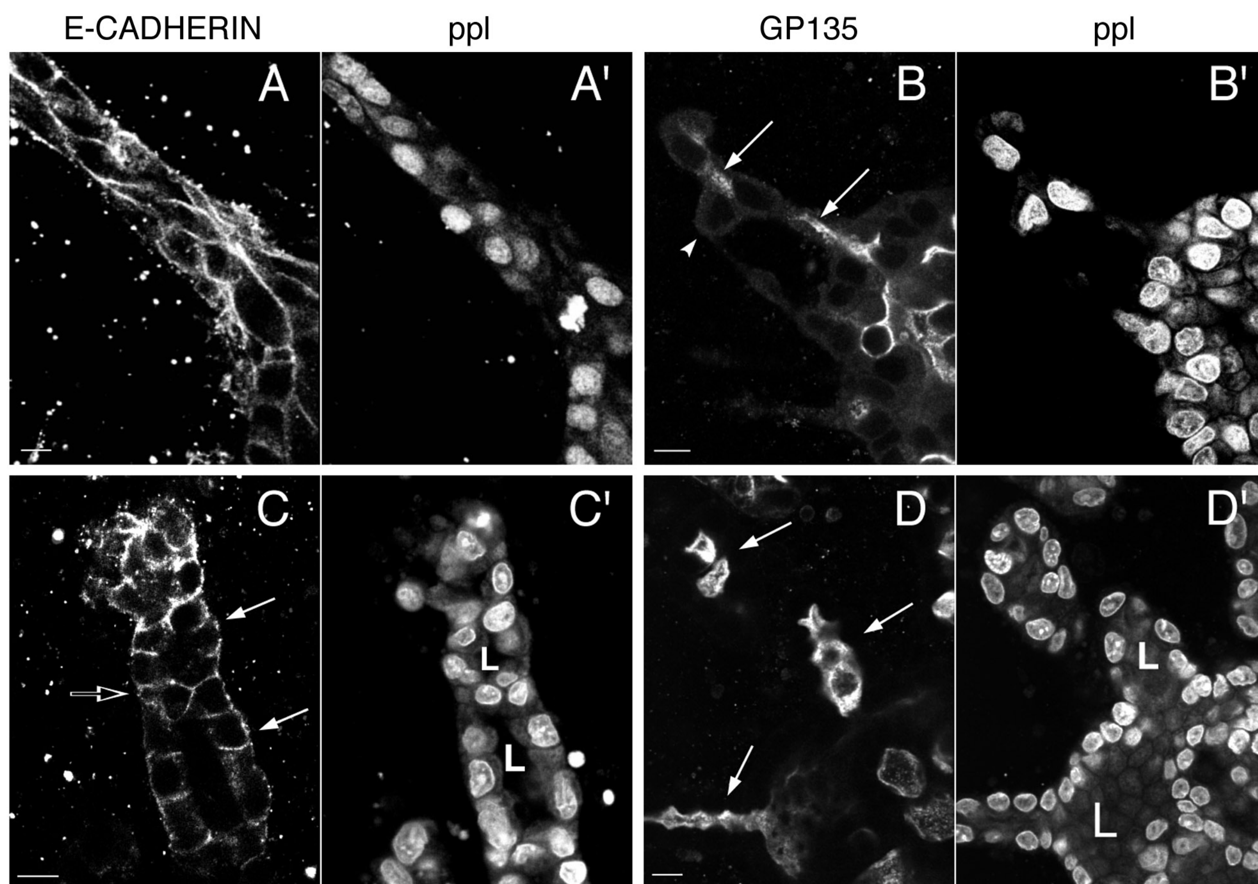

FIG. 3. Apical/basolateral polarity is restored in maturing tubules during *de novo* lumen formation. Single confocal images taken from serial sections through cords and developing tubules are shown. In A–D′ images are split so that staining for E-cadherin (FITC; A and C) or gp135 (FITC; B and D) is in the left half and nuclear staining (ppI; A′–D′) is in the right half. (A and A′) Cells in cords are completely surrounded by E-cadherin. (B and B′) During the development of cords gp135 staining reappears and is strictly localized to regions of cell–cell contact that represent newly forming lumenal membrane (closed arrows). Gp135 staining is absent on membranes in contact with extracellular matrix (B, arrowhead). (C and C′) Tubules develop from cords by *de novo* lumen formation. Cells in regions of developing tubules without lumens (C, open arrow) are completely surrounded by E-cadherin. In lumen-containing regions of tubules E-cadherin staining is basolaterally polarized (C, closed arrows). (D and D′) Gp135 staining in lumen-containing tubules shows that apical membrane polarity is fully restored (closed arrows). L, lumen. Bar, 10 μm.

(Fig. 4B, arrowhead). This suggests that desmosomal adhesion contributed to the maintenance of the cell–cell contacts between the extending cell and the cyst. DpI/II were absent from the lumenal membrane of the extending cell (data not shown), indicating that, similar to E-cadherin, basolateral polarization of cell surface dpI/II is maintained at this stage of tubulogenesis. During the formation of chains dpI/II were localized in large intracellular pools in cells of the chain (Fig. 4C, arrows). Punctate spots of dpI/II were also found between cells of chains (Fig. 4C, arrowhead) but were often difficult to detect (not shown). This suggests that dpI/II is localized to desmosomal plaques at membrane borders between cells of chains. However, the increase in intracellular dpI/II does not result in a corresponding increase in the number or size of desmosomal plaques. The apparent increase in the total immunofluorescence signal may be due to increased accessibility of antibody to nonplasma-membrane dpI/II. However, it may also reflect an upregulation of protein synthesis in cells in which motility is stimulated by SF/HGF. Finally, once polarized lumen-containing tubules are formed dpI/II are localized in punctate spots at lateral membrane cell–cell borders and are not detected in intracellular pools (data not shown).

The TJ is located at the apical-most aspect of the lateral membrane of polarized epithelial cells where it separates the apical and basolateral membrane domains and forms a selective permeability barrier to paracellular transport (Gumbiner, 1987; Rodriguez-Boulan and Nelson, 1989). ZO-1, a cytoplasmic plaque component of the TJ, is found exclusively at the tight junction of polarized epithelial cells

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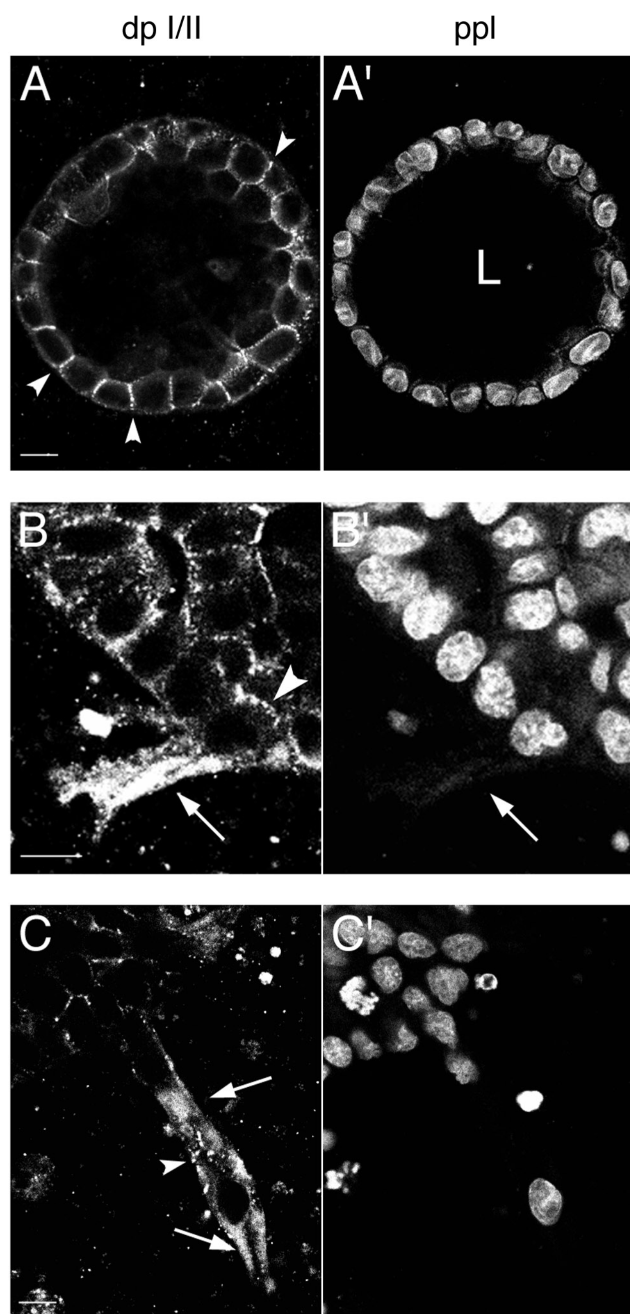

FIG. 4. Desmosomal plaque proteins appear in large intracellular pools during early stages of tubule development. Each image pair is a representative single confocal section taken from serially sectioned samples that were scanned simultaneously for FITC and ppI. The images are split, with dpI/II shown on the left side and nuclei on the right side. (A, A′) In polarized cysts dpI/II are localized to discrete spots along lateral cell membranes (arrowheads). (B, B′) Arrows in B and B′ are aligned to show that during formation of extensions dpI/II appears in large intracellular pools in the region of the cell extending into the extracellular matrix. DpI/II are retained in punctate complexes along membranes at cell–cell borders (arrowhead). (C, C′) Cells in chains contain intracellular pools of dpI/II (arrows). Punctate spots of dpI/II also appear at cell–cell borders between cells of the chain (arrowhead). L, lumen. Bar, 10 μm.

(Siliciano and Goodenough, 1988; Stevenson *et al.*, 1988, 1986). In polarized MDCK cell cysts, ZO-1 is localized at the apical-most aspect of lateral cell–cell borders (Fig. 5A, arrows). By summing multiple confocal sections through a cyst and creating a projection of the images starting above the level of the bottom nuclei (Fig. 5B″) we found that ZO-1 forms an apical "net" (Fig. 5B′) that faces the lumen and rings the apical aspect of each cell of the cyst (Fig. 5B). During the extension stage of SF/HGF-induced morphogenesis, ZO-1 remains at the lumenal aspect of lateral membranes of cells extending from the cyst (Fig. 5C, arrow). Note that the gain of the image in Fig. 5C was increased after collection in Adobe Photoshop to make it possible to detect background staining and visualize the connection of the unlabeled cell extension to the cyst. As tubule formation continues, ZO-1 localizes to regions of membrane that link cells into single-file chains (Fig. 5D, arrows), indicating that ZO-1 remains at sites of cell–cell contact throughout the formation of chains. During *de novo* lumen formation, ZO-1 localizes to the apical-most aspect of lateral membrane borders between cells surrounding the newly formed lumen (Fig. 5E, arrowhead), suggesting that at this stage of tubule development the apical polarization of the TJ is reestablished. In regions of the developing tubule where lumen formation is more advanced, ZO-1 staining outlines the apical membrane, reminiscent of ZO-1 localization at the apical-most aspect of lateral membranes in polarized cysts (Fig. 5E, arrow). The localization of ZO-1 throughout tubulogenesis at membranes involved in cell–cell contact suggests that ZO-1-containing junctions contribute to the maintenance of cell–cell adhesion during all stages of tubule morphogenesis. Concomitant with repolarization of the tubule epithelium, ZO-1 demarcates lumenally polarized tight junctions that separate the apical and basolateral plasma membrane domains. Taken together, our results show that the treatment of MDCK cell cysts with SF/HGF causes disparate changes in the distribution of E-cadherin, desmoplakins, and ZO-1 and suggest that adhesion through each of these cell–cell junctions is differentially regulated during SF/HGF-induced tubulogenesis.

DISCUSSION

Numerous studies have increased our understanding of molecules that induce tubulogenesis and regulate branching activity (Montesano *et al.*, 1991a; Sakurai *et al.*, 1997a,b; Sariola and Sainio, 1997; Sutherland *et al.*, 1996; Vainio and Muller, 1997). However, the morphogenetic mechanisms by which cells reorganize membrane subdo-

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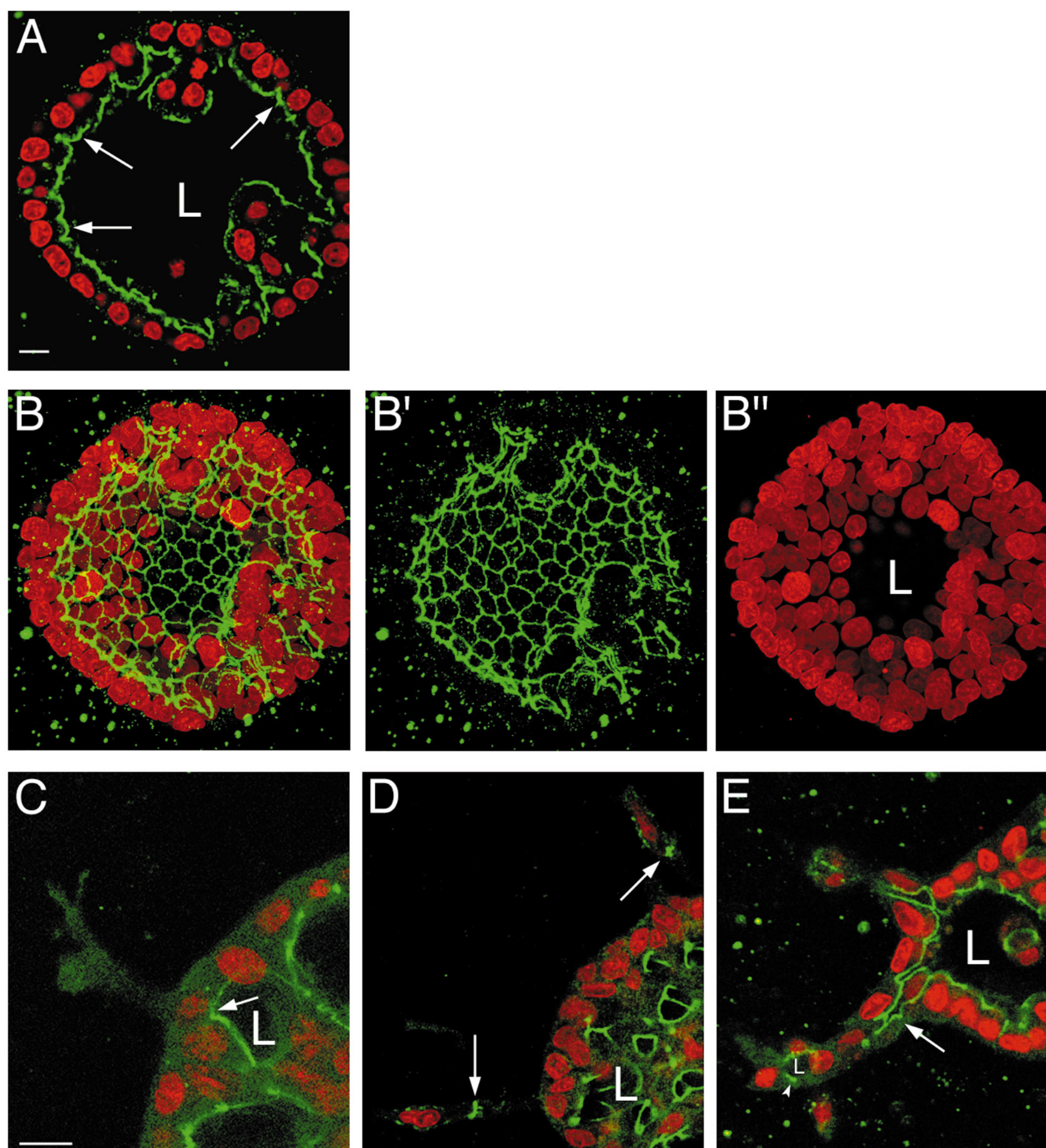

FIG. 5. The tight junction marker, ZO-1, localizes at sites of cell–cell contact at all stages of tubule formation. In A–E serial confocal sections were collected from samples at different stages of tubule morphogenesis. FITC (ZO-1, green) and ppI (red) for all images were scanned simultaneously. A,C, D, and E are representative single confocal sections. B, B′, and B″ each show a projected sum of 13 2-μm serial sections from the same sample as in A. (A) In a cross section through a polarized cyst arrows show that ZO-1 is apically polarized. (B, B′, and B″) The image in B shows the overlay of projections in B′ and B″ that were split to show ZO-1 on the left and nuclei on the right. A net of ZO-1 staining is detected in B′. B″ shows that sections for these projections start above the level of the nuclei of cells that are in the center of the image. The double image in B shows that ZO-1 staining is above the level of the nuclei, demonstrating that ZO-1 is localized to the apical-most aspect of lateral cell–cell borders and forms a ring around each cell of the cyst. (C) In extensions, ZO-1 is detected at the remaining apical membrane facing the lumen of the cyst (arrow). (D) During the formation of chains, ZO-1 is concentrated at cell–cell borders between cells of the chains (arrows). (E) During the development of a lumen-containing tubule ZO-1 remains at cell–cell borders and localizes to the apical-most aspect of lateral membranes of both a newly formed lumen (arrowhead) and a more mature lumen (arrow). L, lumen. Bar, 10 μm. Bar in A applies to B, D, and E.

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mains and cell–cell contacts during tubulogenesis have not previously been well characterized.

Distinct Stages in Tubulogenesis

Based on a detailed three-dimensional analysis we have defined four stages in the development of tubules from MDCK cell cysts (see Fig. 1) in which we have identified unique arrangements of marker proteins for apical/basolateral polarity and cell junctional complexes. These stages define the morphogenetic mechanisms for tubule formation in this system. In the first phase of SF/HGF-induced tubule formation, the extension stage, individual cells protrude into the surrounding collagen matrix while remaining attached to the cyst. Apical and basolateral membranes are morphologically altered but cell polarity and cell junctions are maintained. Some individual scattered cells are detected in these cultures but do not appear to contribute to tubule development. The second stage, chain formation, is characterized by the appearance of single-file linear or branched chains of cells in which an apical membrane marker (gp135) is absent or intracellular, a basolateral membrane marker (E-cadherin) has a nonpolar distribution around the entire cell surface, desmosomes (dpI/II) are predominantly localized in intracellular pools, and a TJ marker (ZO-1) is highly localized to the small regions of cell–cell contact between cells in the chain. During the third stage, cord formation, chains of cells thicken into cords in which apical polarity is again observed while the basolateral marker protein, E-cadherin, remains randomly distributed. *De novo* lumen formation occurs at sites of cell–cell contact where apical membrane markers are located. Small lumens begin to appear in segregated regions along the length of the developing tubule. ZO-1 localizes to apical cell–cell contact points and basolateral repolarization is initiated in cells surrounding the nascent lumens. In the final stage of tubulogenesis, tubule maturation, individual lumens coalesce, enlarge, and become continuous with the lumen of the cyst. Apical and basolateral membranes of cells of the tubule become clearly polarized and the arrangement of cell junctions that normally is found in polarized epithelial cells is completely restored.

Our analysis has yielded at least two new and surprising insights into the mechanisms of tubulogenesis, which are: (i) a novel model of cell and membrane subdomain rearrangement during tubulogenesis and (ii) differential regulation of junctional proteins.

A Novel Model of Tubulogenesis

The pattern of cell rearrangements and intercellular migration that we observe is clearly different from that previously proposed in both the outpouching and the two-step dissociation/reassociation models of tubulogenesis. A key prediction of the outpouching model is that tubule lumens would remain continuous with the cyst lumen at all stages of tubule development (Gilbert, 1994). In fact, our results demonstrate that tubule lumens are initially discontinuous, forming *de novo* at discrete regions along the length of developing tubules. In addition, we observed that newly formed tubule lumens do not necessarily connect to the lumen of the cyst. A second prediction of the outpouching model is that cells would retain their polarity, as they would continue to line a lumen at all stages of tubule formation. This is contrary to our observation that the cells undergo a transient loss of polarity. Therefore, an outpouching model does not explain our observed stages of tubulogenesis. The two-step dissociation/reassociation model predicts that we would have seen aggregates of two or more cells that were detached from cysts forming structures such as chains, cords, or tubules. We did not observe such structures. Rather, the chains, cords, and tubules that we observed were always in contact with cysts. Therefore, tubules did not arise by a complete loss of cell–cell contact followed by reassociation, as proposed by Thiery and Boyer (1992). This was consistent for over 250 cysts that we stimulated with SF/HGF and analyzed by complete serial sections.

We propose, therefore, a novel model of tubulogenesis that includes the following morphogenetic mechanisms: (1) stimulation of cell migration is the first step in tubulogenesis, (2) apical/basolateral polarity is transiently lost and is restored concomitant with lumen formation, (3) discontinuous tubule lumens form *de novo* in regions of developing tubules, and (4) cell–cell contacts are retained at all stages of tubulogenesis.

A key observation from our results is that tubule formation is initiated by the extension and migration of cells without loss of cell–cell contact. Migration of cells without loss of cell–cell contact has previously been found to be important for organ formation during development *in vivo.* For example, chain migration of neuronal precursors contributes to the formation of the olfactory bulb (Lois *et al.,* 1996). The fundamental role of SF/HGF in this tubulogenesis model system may be to induce cells to migrate out from a polarized epithelium to develop extensions and form a chain. Previous studies have shown that altering cell–substrate contacts by collagen overlay is sufficient to initiate the direct conversion of MDCK cell monolayers into tubulocysts (Schwimmer and Ojakian, 1995; Zuk and Matlin, 1996). In the collagen overlay technique, the conversion of a monolayer of cells into a tubule-like structure may recapitulate the last two stages that we have identified in SF/HGF-induced tubulogenesis, namely the conversion of a chain into a cord and subsequently a cord into a lumen-containing tubule. In contrast to our studies, this direct conversion of a monolayer into a tubule-like structure essentially bypasses the requirement for an inducing molecule such as SF/HGF. Therefore, during tubulogenesis, once SF/HGF induces formation of a chain in contact with collagen, the further formation of a cord and a tubule may be the result of a mechanism intrinsic to the MDCK cells, without requiring further SF/HGF signaling.

We have observed that cells branching from chains and

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tips of tubules also undergo changes in morphology, polarity, and cell–cell contacts that are similar to the changes in the initial cell extensions from cysts (A. Pollack and K. Mostov, unpublished results). In addition, our recent studies of interactions of β-catenin and APC protein during tubulogenesis have shown that mechanisms regulating cell migration are important for both formation of extensions and the later development of mature tubules rather than polyp-like structures (Pollack *et al.,* 1997). Our results suggest that cell migration is an important mechanism at multiple stages of tubule development. Therefore, we propose that a triggering function of SF/HGF may be important, not only for inducing formation of extensions and chains, but also for stimulating elongation of the tips of tubules and initiating cell movements in the development of branches.

Our analysis of cell polarity during tubule development has also contributed to the formulation of a model of tubulogenesis. A key mechanism derived from this analysis is the dynamic regulation of cell polarity during tubulogenesis: cells transiently lose their polarity during the formation of chains, but subsequently regain polarity as they form mature tubules. During tubulogenesis, after initial loss of polarity, apical polarity is reestablished first in regions of cell–cell contact in cords, followed by basolateral repolarization during lumen formation. Previous studies have shown that changes in cell–cell and cell–substrate interactions alter epithelial cell polarity (Rodriguez-Boulan and Nelson, 1989; Vega-Sales *et al.,* 1987; Wang *et al.,* 1990a). For example, when MDCK cysts are first grown in a liquid suspension culture the apical surface of the cells is localized at the outside membrane of the cyst, facing the medium. When recultured in collagen gels, these cells undergo a reversal of polarity without loss of cell–cell contacts. During this process the apical marker gp135 disappears by internalization and degradation and then is resynthesized (Wang *et al.,* 1990b). The loss of gp135 from the transiently nonpolarized cells during SF/HGF-induced tubulogenesis, and the subsequent accumulation of gp135 at the nascent apical surface, may be regulated analogously. Therefore, mechanisms driving changes in epithelial polarity during tubulogenesis may be similar to other systems in which epithelial cell subdomains rearrange. This requires further study. Importantly, our results show that dynamic rearrangement of proteins that are normally polarized to specific membrane subdomains, without a loss of cell–cell contacts, is an important feature of epithelial tubule development. In addition, we provide a perspective of how sequential changes in apical/basolateral polarity fit into the larger context of the morphogenetic process of tubulogenesis.

Differential Regulation of Junctional Proteins during Tubulogenesis

A second new insight demonstrated by our studies is that components of the three types of cell–cell junctions, adherens junctions, desmosomes, and tight junctions, underwent very different patterns of regulation during tubulogenesis: E-cadherin redistributed throughout the cell surface, dpI/II accumulated intracellularly, and ZO-1 was found at regions of cell–cell contact. Although junctional proteins were modified, cell–cell contacts were retained throughout the intricate cell rearrangements during morphogenesis of a tubule from a cyst. The selective regulation of distinct cell–cell junctional components during tubulogenesis contrasts strikingly with results from previous studies in which calcium "switch" or antibody disruption of E-cadherin-based adhesion caused coordinate regulation of adhesion through desmosomes, adherens junctions, and tight junctions (Behrens *et al.,* 1985; Gumbiner and Simons, 1986; Gumbiner *et al.,* 1988; Siliciano and Goodenough, 1988). We suggest that differential regulation of cell–cell junctional proteins, both spatially and temporally, is likely to be crucial to maintain cell–cell contacts during the development of tubules.

We have previously shown that E-cadherin surrounding the cell perimeter in extensions, chains, and cords colocalizes with β-catenin, a protein that is normally associated with the cytoplasmic domain of E-cadherin and is required for E-cadherin-based cell–cell adhesion (Pollack *et al.,* 1997). In addition, we have reported that monolayer cultures of MDCK cells treated with SF/HGF show an increase in E-cadherin synthesis, which correlates with SF/HGF-induced morphogenetic cell rearrangements (Balkovetz *et al.,* 1997). It appears, therefore, that as cells extend and form chains, they produce additional E-cadherin, which is expressed on surfaces not yet in contact with other cells. This E-cadherin is not initially utilized for cell–cell contact, either because it is differentially regulated so that it is not yet competent for cell–cell adhesion, or perhaps simply because no other cell surface is present with which the E-cadherin can interact. As chains are transformed into cords, this E-cadherin is utilized for the establishment of new cell–cell contacts.

During the initial extension formation, ZO-1 remains at the cell surface at the boundary between the apical and the basolateral domains, showing that the fence function of tight junctions remains intact. Next, during the chain stage ZO-1 is localized only to the regions of cell–cell contact. We speculate that this ZO-1, which is much more highly localized to the regions of cell–cell contact than E-cadherin, may play a role in maintaining cell–cell contact during this stage of tubulogenesis. The ZO-1 subsequently relocalizes to the apex of cells, surrounding newly formed lumens. It is interesting to compare our results with observations made in the calcium switch system. There, ZO-1 is initially colocalized with E-cadherin along the length of the region of cell–cell contact. Subsequently, the ZO-1 is segregated to the region of the tight junctions near the apex of the cell (Rajasekaran *et al.,* 1996).

Finally, the desmosome components, dpI/II, are found in large intracellular pools, initially appearing in cells that are stimulated to migrate by SF/HGF. SF/HGF has been found

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to cause loss of desmosomal plaques in MDCK cells during scattering (Stoker and Perryman, 1985) and to disrupt desmosomal contacts in keratinocytes (Watabe *et al.,* 1993). SF/HGF treatment of MDCK cell monolayers also causes accumulation of dpI/II in large intracellular pools during morphogenetic cell rearrangements (A. Pollack and K. Mostov, unpublished observations). These studies have provided evidence that SF/HGF induces either internalization or new synthesis and cytoplasmic retention of dpI/II. Taken together with the results of our studies, we suggest that modulating desmosomal adhesion by removal of dpI/II from the cell surface is important for morphogenetic cell rearrangements during tubulogenesis.

Our studies suggest that SF/HGF selectively modulates, rather than disrupts, cell–cell adhesion during tubulogenesis. The segregation of SF/HGF's effects on components of adherens junctions, desmosomes, and tight junctions may be critical for regulating the formation of lumen-containing tubules. During metanephric kidney development *in vivo,* cells do not appear to dissociate as new structures are formed (Vestweber *et al.,* 1985; Garrod and Fleming, 1990). Formation of ureter and tubular structures in the developing kidney coincides with the expression of E-cadherin- (Vestweber *et al.,* 1985) and desmoplakin-containing desmosomes (Garrod and Fleming, 1990) at epithelial cell–cell borders. Patterns of desmoplakin expression and the structure of the desmosomes change during kidney development, indicating that lability of desmosomes is important for kidney morphogenesis (Burdett, 1993; Garrod and Fleming, 1990). These *in vivo* studies suggest that regulation of cell–cell adhesion molecules is important early in kidney development. Differential regulation of cell–cell junctional proteins in both space and time may allow the adhesive properties of specific areas of individual cells to be modulated so that cells "let go" and reconnect as they rearrange. Previously, components of cell–cell junctions have been shown to be regulated by phosphorylation (Behrens *et al.,* 1993; Matsuyoshi *et al.,* 1992; Nigam *et al.,* 1991; Shibamoto *et al.,* 1994; Volberg *et al.,* 1992). Investigation into a role of SF/HGF-induced phosphorylation as a mechanism of differential regulation of cell–cell adhesion during tubulogenesis requires future study.

During tubulogenesis, specific modulation of adhesion and polarity may enable a developing system to restructure without having to completely redifferentiate. Regulating the extent to which adhesion and polarity are altered during morphogenesis may prime a developing system to rapidly restore or acquire epithelial function. In morphogenetic processes such as wound repair (Bement *et al.,* 1993; Madara, 1990) it is critical that epithelial cell rearrangements occur with a minimal disruption of polarity and adhesion in order to maintain the functional intactness and permeability characteristics of the epithelial tissue. Further insight into the interplay between the actions of SF/HGF and components of systems that regulate cell–cell and cell–substrate adhesion and polarity may provide a key to understanding the seemingly disparate abilities of SF/HGF to induce epithelial cells to develop into cohesive structures rather than to scatter or invade.

In conclusion, in this study we have carefully analyzed the temporal and spatial regulation of cell polarity and cell–cell adhesion proteins subsequent to induction of tubulogenesis from a polarized epithelium to understand the mechanisms of cell rearrangements in the development of lumen-containing tubules. We suggest a novel multistage model of tubulogenesis in which SF/HGF stimulates initial cell migration and remodeling of cell polarity but differentially regulates cell–cell junctional proteins, such that cell–cell adhesion is maintained throughout cell rearrangements. In addition, we propose that an intrinsic program of epithelial repolarization may be critical to the formation of mature tubules. Our study is the first report of a detailed examination of cell rearrangements combined with analysis of the accompanying changes in cell adhesion, junctions, and polarity that occur during epithelial tubulogenesis in any model system. These results provide a framework for future investigations of cell movements, polarity, and junctions during morphogenetic cell rearrangements of tubulogenesis *in vivo* and in other *in vitro* systems that reflect this developmental process in a variety of organs.

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