Activated extracellular signal-regulated kinases are necessary and sufficient to initiate tubulogenesis in renal tubular MDCK strain I cell cysts

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Hellman, Nathan E., Andres J. Greco, Katherine K. Rogers, Chitra Kanchagar, Daniel F. Balkovetz, and Joshua H. Lipschutz. Activated extracellular signal-regulated kinases are necessary and sufficient to initiate tubulogenesis in renal tubular MDCK strain I cell cysts. Am J Physiol Renal Physiol 289: F777–F785, 2005. First published May 17, 2005; doi:10.1152/ajprenal.00429.2004.—A classic in vitro model of renal cyst and tubule formation utilizes the Madin-Darby canine kidney (MDCK) cell line, of which two strains exist. Most cyst and tubule formation studies that utilized MDCK cells have been performed with MDCK strain II cells. MDCK strain II cells form hollow cysts in a three-dimensional collagen matrix over 10 days and tubulate in response to hepatocyte growth factor, which increases levels of active (phosphorylated) ERK1/2. In this study, we demonstrate that MDCK strain I cells also form cysts when grown in a collagen matrix; however, MDCK strain I cell cysts spontaneously initiate the primary steps in tubulogenesis. Analysis of time-lapse microscopy of both MDCK strain I and strain II cell cysts during the initial stages of tubulogenesis demonstrates a highly dynamic process with cellular extensions and retractionss occurring rapidly and continuously. MDCK strain I cell cysts can spontaneously initiate tubulogenesis mainly because of relatively higher levels of active ERK in MDCK strain I, compared with strain II, cells. The presence of either of two distinct inhibitors of ERK activation (UO126 and PD09059) prevents tubulogenesis from occurring spontaneously in MDCK strain I cell cysts and, in response to hepatocyte growth factor, in strain II cell cysts. The difference between MDCK strain I and strain II cell lines is likely explained by differing embryological origins, with strain I cells being of collecting duct, and hence ureteric bud, origin. Ureteric bud cells also have higher levels of active ERK and spontaneously tubulate in our in vitro collagen gel system, with tubulogenesis inhibited by UO126 and PD09059. These results suggest that a seminal event in kidney development may be the activation of ERK in the mesonephric duct/ureteric bud cells destined to form the collecting tubules.

Madin-Darby canine kidney cells; ureteric bud

EPITHELIAL ORGANS SUCH AS the kidney, lung, and mammary gland originate from two basic types of “building blocks,” cysts and tubes (9, 16, 26, 30, 33, 34, 39). The kidney is particularly well suited for the study of cyst and tubule formation and, like many other epithelial organs, forms as a result of a reciprocal interaction between epithelium and mesenchyme (15). The epithelial component during metanephric kidney development is the ureteric bud, and the mesenchymal component is the metanephric mesenchyme. During metanephric kidney development, the tip of each ureteric bud branch induces development of renal vesicles, which are epithelial cysts of metanephric mesenchyme origin that undergo morphogenetic transformation to eventually form the tubular structures from the glomerulus to the distal tubule. The ureteric bud ultimately forms the collecting tubules of the kidney (1, 8). The factors that initially induce the ureteric bud, also known as the metanephric diverticulum, to form and grow from the lower end of the mesonephric duct, also called the Wolffian duct, are not well understood.

Because of the complexity of organogenesis [the human kidney is composed of more than 20 cell types and one million nephrons (27, 36)] and the transitory nature of cyst and tubule formation, it is difficult to study these processes in vivo. Relatively little, therefore, has been known about cyst formation before the development of an in vitro assay. The MDCK cell line, of which two strains exist, was derived from the kidney tubules of a normal cocker spaniel in 1958 (13, 18) and, for the past several decades, has been one of the most widely used systems for studying important and fundamental issues in epithelial cell biology (38). When Madin-Darby canine kidney (MDCK) cells were seeded within a three-dimensional collagen matrix over 10 days, they formed structures that were characterized by a polarized epithelium surrounding a fluid-filled space, apical microvilli, a solitary cilium, and apical tight junctions (21, 22), meeting the most rigorous definition of “cysts” (20). Exposure of preformed MDCK cysts to hepatocyte growth factor (HGF) caused the cysts to develop branching tubules (23) in a process that resembles renal tubulogenesis in vivo (36). The vast majority of studies examining cyst and tubule formation using MDCK cells were performed with strain II cells (16, 26, 32).

MDCK strain I cells, which were derived from an early passage of the cell population, and MDCK strain II cells, which predominate in later passages, are likely to have originated from separate nephron segments (31, 40). Although it is unrealistic to expect a complete coidentity with separate nephron segments, because the MDCK cell line is established and adapted to culture conditions, MDCK strain I cells were determined to be of cortical collecting duct cell origin based on their high electrical resistance, their responsiveness to epinephrine and vasoressin, and the absence of more proximal marker

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enzymes, such as alkaline phosphatase and γ-glutamyl transferase (31). MDCK strain II cells resemble more proximal renal tubular epithelial cells because the short-circuit current is not stimulated by epinephrine and vasopressin; these cell monolayers possess a low electrical resistance, and alkaline phosphatase and γ-glutamyl transferase are present (31). Another major difference between MDCK strain I and strain II cells that is directly related to the difference in transepithelial resistance is the presence of higher levels of active (phosphorylated) ERK1/2 in MDCK strain I, compared with strain II, cells (17). In vivo, much higher levels of active ERK1/2 were also observed in the collecting ducts of the human nephron (19).

Studies using MDCK strain II cells grown in a collagen matrix until the cyst stage and induced with HGF have shown that tubulogenesis consists of two morphologically defined stages: a partial epithelial-mesenchymal transition (p-EMT) and subsequent redifferentiation (26, 27, 30). The signaling pathway demonstrated to play a role in the p-EMT stage of tubulogenesis is the MAPK pathway of Raf-MEK-ERK, one of the many signaling cassettes that is activated on binding of HGF to its tyrosine kinase receptor, c-Met (2, 26, 28). This paper will focus on the initial p-EMT stage of tubulogenesis and the morphological manifestations of long cytoplasmic extensions and small chains of cells extending off the basolateral surface of the cyst cells. p-EMT occurs within the first 24 h after HGF stimulation and requires cell de-differentiation and loss of apical-basolateral polarity. The subsequent redifferentiation stage, occurring 24–72 h after HGF stimulation, depends on matrix metalloproteinases (MMPs) and involves the generation of more mature cords of cells and eventually true tubules containing fully formed lumens with well-defined polarity (26, 27, 30). ERK inhibition using the pharmacological inhibitors UO126 and PD09059 prevented HGF-induced p-EMT, and therefore tubulogenesis, in MDCK strain II cell cysts, whereas growth of an MDCK strain II cell line stably resistant is the presence of higher levels of active (phosphorylated) ERK1/2 in MDCK strain I, compared with strain II, cells (17). In vivo, much higher levels of active ERK1/2 were also observed in the collecting ducts of the human nephron (19).

Given that ERK expression is necessary and sufficient for initiation of tubulogenesis (26) and that MDCK strain I cells express higher levels of active ERK (17), we hypothesized that MDCK strain I cells would spontaneously undergo at least the early stages of tubule formation. In this study, we demonstrate that MDCK strain I cells do, in fact, spontaneously initiate tubulogenesis in an ERK-dependent manner. Because MDCK strain I cells are likely of collecting duct, and hence ureteric bud, origin, we examined a ureteric bud cell line and found that ureteric bud cells also have high levels of active ERK. Ureteric bud cells cysts spontaneously initiate tubulogenesis, and tubulogenesis can be inhibited by inhibitors of ERK activation. The unique MDCK system of cell lines derived from the same genetic background, but differing with respect to their expression levels of active ERK, provides a powerful tool for investigating the role of the MAPK signaling pathway in cyst and tubule formation and suggests a possible model for renal branching morphogenesis.

MATERIALS AND METHODS

Cystogenesis and tubulogenesis. Low-passage MDCK strain I (obtained from Karl Matlin, University of Cincinnati) and strain II (obtained from Keith Mostov, University of California, San Francisco) cells were used between passages 3 and 10. The immortalized ureteric bud cell line was generated from the Immortomouse and was a kind gift from Jonathan Barasch (Columbia University, New York, NY) (3). Cells were cultured as previously described and maintained in modified MEM containing Earl’s balanced salt solution and glutamine supplemented with 5% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (17). For growth of cells in collagen gels, the MDCK cells were trypsinized and triturated into a single-cell suspension of 2 × 10⁶ cells/ml in a type I collagen solution as described previously (16). Medium was changed every 4 days. After 10 days, recombinant human HGF at 100 ng/ml (generously provided by R. Schwall, Genentech, South San Francisco, CA) was added to the medium surrounding the collagen gels. UO126 (Promega) and PD09059 (Sigma), both inhibitors of MEK, were used as described in RESULTS.

Microscopy. Cells in collagen gel were rinsed in PBS and fixed for 30 min with 4% paraformaldehyde after digesting in 100 U/ml collagenase (Sigma) for 10 min at 37°C as previously described (16). Nonspecific binding sites were blocked, and the cells were permeabilized using 0.7% fish skin gelatin and 0.025% saponin. Samples were placed in medium containing 1:50 dilution Alexa 594 phalloidin (Molecular Probes, Eugene, OR). After extensive washing, the samples were postfixed with 4% paraformaldehyde and mounted. Images were collected using a Qicam 1394 camera attached to a Nikon Eclipse E600 immunofluorescence microscope (Nikon, Melville, NY).

Western blot analysis. Western blot analysis was performed as described previously (17). For total ERK analysis, filters were probed with goat-anti-ERK1 polyclonal IgG (1:1,000) (Santa Cruz Biotechnology). This antibody recognizes both ERK1 and ERK2 in MDCK cell lysates. Filters were then probed with horseradish peroxidase-labeled donkey anti-goat at 1:15,000 dilution. For active ERK (MAPK) analysis, filters were probed with phospho-p44/42(ERK1/2) MAPK antibody (1:1,000, Cell Signaling Technologies). Filters were then probed with horseradish peroxidase-labeled goat anti-rabbit at 1:15,000 dilution. Filters were developed using a SuperSignal West Femto maximum sensitivity substrate kit (Pierce) and visualized on Kodak X-OMAT film (Eastman Kodak, Rochester, NY). Western blot experiments were performed at least three times.

Time-lapse microscopy. MDCK strain I and II cells were grown in collagen matrix as described above. On day 6 for MDCK strain I cell cysts and day 11 for MDCK strain II cell cysts, images were captured every 3 min using light microscopy in the presence and absence of recombinant HGF at 100 ng/ml over an ~15-h period. Images were captured using an Evolution QEi camera (Media Cybernetics, Silver Spring, MD) attached to a Nikon TE300 microscope and processed with Image Pro Plus software (Media Cybernetics, Silver Spring, MD).

Cyst measurement and statistical analysis. Images of the first 25 cysts seen in the collagen gel were captured during immunofluorescence microscopy as described above. The cysts were identified, for each condition, first in the right margin and then as the stage was moved from right to left. The number of extensions per cyst and the diameter of the cysts were measured, and the mean and standard deviation were determined using Excel software (Microsoft). Comparison of the means was performed with the Student’s t-test, and comparison of percentages was performed with χ² and Fisher’s exact probability tests (both part of the Excel Software package; Microsoft).
RESULTS

MDCK strain I cells spontaenously initiate tubulogenesis. On the basis of the recent findings that ERK is involved in the initial p-EMT stage of renal tubulogenesis (26) and MDCK strain I cells express higher levels of activated ERK than MDCK strain II cells (17), we hypothesized that MDCK strain I cells would spontaneously initiate tubulogenesis. To investigate this, MDCK strain I and strain II cells were grown in a three-dimensional collagen matrix in the absence of HGF (Fig. 1). Both strain I and strain II cells formed spherical cysts, which were fixed and processed for immunofluorescence using rhodamine-tagged phalloidin (which stains actin) on days 5, 7, 9, and 11. Although the MDCK strain II cell cysts did not tubulate, many of the strain I cysts spontaneously formed one to several cellular extensions and chains of cells, characteristic of the p-EMT stage of tubulogenesis, although rarely, if ever, cords of cells or actual tubules containing lumens, which are characteristic of the redifferentiation stage of tubulogenesis (Fig. 1A). Not all MDCK strain I cell cysts were observed to contain extensions, and MDCK strain I cell cysts grew faster and larger than MDCK strain II cell cysts.

To quantify these observations, 25 cysts were chosen in a random manner and cyst diameter and number of extensions per cyst were determined. For the purposes of this analysis, “extensions” refers to both cellular extensions and chains of cells, which are morphological representations of p-EMT and have been shown to be dependent on the presence of active ERK (26). True tubules containing lumens, which form during the redifferentiation stage of tubulogenesis, were not observed in any of the MDCK strain I or II cell cysts.

Fig. 1. Madin-Darby canine kidney (MDCK) strain I cell cysts spontaneously initiate tubulogenesis and grow more rapidly than MDCK strain II cell cysts. A: comparison of a representative MDCK strain I cell cyst (left) and strain II cell cyst (right) at 11 days in the absence of hepatocyte growth factor (HGF). Extensions are seen in the MDCK strain I cell cyst (arrow). Both cysts were visualized using rhodamine-tagged phalloidin, which stains actin, and are shown at identical magnification. Bars = 50 μm. Note that the aperture of the microscope was narrowed to focus just on the cyst to decrease stray light. B: bar graph demonstrating the increased cyst diameter in MDCK strain I cell cysts in the absence (black bar) vs. presence (gray bar) of HGF. C: bar graph of percentage of cysts with at least one extension in MDCK strain I cell cysts in the absence (black bars) or presence (gray bars) of HGF. There was a significant change after HGF stimulation only in the MDCK strain II cell cysts. For B and C, 25 cysts for each condition were randomly chosen, and the resultant measurements and percentages were used for statistical analysis. Comparison of means was performed with Student’s t-test, and comparison of percentages was performed with χ² and Fisher’s exact probability tests (Excel Software package, Microsoft).

Fig. 2. HGF stimulates tubulogenesis in MDCK strain II but not in strain I cell cysts. A: representative MDCK strain I and MDCK strain II cell cysts at 11 days in the presence of HGF for 24 h. B: bar graph showing no difference in the average cyst diameter of MDCK strain I cell cysts in the absence (black bar) vs. presence (gray bar) of HGF. C: bar graph of percentage of cysts with at least one extension in MDCK strain I and strain II cell cysts grown in the presence of HGF. There was a significant change after HGF stimulation only in the MDCK strain II cell cysts. For B and C, 25 cysts for each condition were randomly chosen, and the resultant measurements and percentages were used for statistical analysis. Comparison of means was performed with Student’s t-test, and comparison of percentages was performed with χ² and Fisher’s exact probability tests (Excel Software package, Microsoft).
the redifferentiation stage of tubulogenesis, are dependent on MMPs and not on active ERK (26). Strain I cysts grew faster than strain II cysts (Fig. 1B) with a statistically significant difference in cyst diameter apparent as early as day 7. Approximately 40–70% of the strain I cysts contained at least one extension, whereas virtually none of the strain II cysts displayed extensions (Fig. 1C).

Unlike MDCK strain II cell cysts, MDCK strain I cell cysts do not undergo further tubulogenesis in response to HGF. HGF induces tubulogenesis in MDCK strain II cell cysts (30). To determine whether HGF would induce tubulogenesis in MDCK strain I cell cysts, recombinant HGF was added to MDCK strain I and strain II cell cysts for 24 h at days 5, 7, 9, and 11. Immunofluorescence staining revealed that MDCK

Fig. 3. Time-lapse photography demonstrating MDCK tubulogenesis. A: MDCK strain I cell cyst in the absence of HGF stimulation demonstrates spontaneous tubulogenesis. An extension (white arrowheads) was observed to be present at the onset of the experiment but subsequently retracted after ~3 h (recombinant HGF was added to the medium 1 h before the time-lapse imaging began). Other extensions (black arrowheads) were highly dynamic, extending and retracting over the entire 15-h period. B: MDCK strain II cell cyst after HGF stimulation. The first extensions are noted to occur at ~3 h after HGF stimulation. Black and white arrowheads indicate examples of extensions that were observed to extend and subsequently retract during the course of this experiment. Time-lapse microscopy was performed for 3 cysts in each condition, and results were very similar. Representative cysts are shown.
strain II cell cysts displayed a vigorous tubulogenic response to HGF, as we and others have shown (16, 23, 30), whereas MDCK strain I cell cysts initiated tubulogenesis at approximately the same rate as seen in the absence of HGF (Fig. 2A). There was also no difference in MDCK strain I cell cyst diameter following addition of HGF (Fig. 2B). Further HGF stimulation of MDCK strain I cell cysts for periods of up to 72 h also did not result in any additional response (data not shown). Morphometric analysis of the cysts was performed. Nearly 70% of MDCK strain II cell cysts responded to HGF stimulation by forming extensions within the first 24 h, whereas there was a nonsignificant increase in the percentage of strain I cysts with extensions following HGF stimulation (36 vs. 44%, Fig. 2C). In addition, strain II cell cysts stimulated by HGF displayed significantly more extensions (average no. of extensions per cyst was 5.53), whereas strain I cysts had fewer extensions per cyst (average was 2.73) in either the absence or presence of HGF (data not shown).

The results in Fig. 3B are similar to those shown as supplemental data in a recent review article on tubulogenesis (27). These data strongly support the idea that the reason only ~40–70% of the MDCK strain I cell cysts showed evidence of extensions with immunofluorescence microscopy was due to the transient and dynamic nature of this process. HGF treatment of MDCK strain I cell cysts observed under time-lapse microscopy displayed no increase in extension formation compared with non-HGF-treated MDCK strain I cell cysts (data not shown).

ERK is required for initiation of tubulogenesis in both MDCK strain I and strain II cells. From recent studies showing a role for active ERK1/2 in MDCK strain II cell tubulogenesis (26) and the presence of active ERK in MDCK strain I cells (17), we hypothesized that ERK inhibition would prevent the spontaneous initiation of tubulogenesis observed in MDCK strain I cell cysts. To this end, both MDCK strain I and II cysts were incubated in the presence or absence of either UO126 or PD09059 (Fig. 4), both of which inhibit MEK, which is the upstream activator of ERK (17). As previously demonstrated (26), the HGF-induced tubulogenesis in MDCK strain II cell cysts was completely prevented by UO126 when added at the same time as HGF (data not shown). When added for a 24-h period, UO126 did not cause obvious cyst toxicity or a de-

Fig. 4. ERK is necessary for initiation of tubulogenesis in MDCK strain I cell cysts. MDCK strain I cell cysts were fixed and stained after 6 days of growth in the collagen matrix. Cysts were incubated for 24 h in the presence of 10 μM of the ERK inhibitor UO126 (UO). A: MDCK strain I cell cysts grown in the presence of UO126 do not spontaneously initiate tubulogenesis. MDCK strain I cell cysts grown in the presence of both HGF and UO126 also do not spontaneously initiate tubulogenesis (data not shown). B: MDCK strain I cells grown in the continuous presence of PD09059 (PD; 50 μM). For C, 25 cysts for each condition were randomly chosen, and the resultant measurements were used to determine the statistical mean and SD. Comparison of means was performed with Student’s t-test (Excel Software package, Microsoft).
crease in cyst size; however, when MDCK strain II cells were plated in the continuous presence of UO126, no cysts developed even after 10 days (data not shown). In MDCK strain I cell cysts grown in the presence of UO126 for 24 h, spontaneous initiation of tubulogenesis was blocked in both the absence (Fig. 4A) and presence of HGF (data not shown). UO126 did not appear to have an overtly cytotoxic effect on MDCK strain I cell cysts when present at the concentrations used for a 24-h period (Figs. 4A). Incubation with UO126 for up to 6 days (Fig. 4B) did allow for the growth of cysts, but they were much smaller in size and were characterized by a lack of spontaneous extension formation. Quantification of the data from MDCK strain I cell cysts grown in the presence of UO126 and another MEK inhibitor, PD09059, showed that both compounds prevented the spontaneous initiation of tubulogenesis (Fig. 4C). Together, these studies show that ERK1/2 activation is required for the spontaneous initiation of MDCK strain I tubulogenesis, as is for HGF-induced MDCK strain II tubulogenesis (26).

Ureteric bud cells express activated ERK. Given that MDCK strain I cells express active ERK and are likely of ureteric bud origin, we examined expression of active ERK in a ureteric bud cell line. The ureteric bud cell line was isolated from mice transgenic for the T antigen. These cells express epithelial and ureteric specific markers, which identifies them as authentic ureteric bud cells (3). Western blot analysis of ureteric bud cell lysate using antibodies against active (phosphorylated) ERK showed abundant active ERK, comparable to levels seen in MDCK strain I cells (Fig. 5A). The presence of phosphorylated ERK was confirmed by incubation of ureteric bud cells with ERK inhibitors, which led to a marked diminution in the levels of active (phosphorylated) ERK, while having minimal effect on total ERK levels (Fig. 5B).

Ureteric bud cell cysts that spontaneously initiate cystogenesis and tubulogenesis are inhibited by inhibitors of ERK activation. Ureteric bud cells formed cysts and spontaneously initiated tubulogenesis when grown in a collagen matrix (Fig. 6A). Tubulogenesis was inhibited by PD09059, and the inhi-

![Fig. 5](http://ajprenal.physiology.org/)
BIBLIOGRAPHIC FIGURE 6. Ureteric bud cell cysts spontaneously tubulate, and tubulogenesis is inhibited by inhibitors of ERK activation. A: comparison of ureteric bud cells grown for 8 days in a collagen matrix in the absence (left) and presence of PD09059, an inhibitor of ERK activation (right). Extensions/tubules are seen in the untreated ureteric bud cell cyst. Cysts were visualized using rhodamine-tagged phalloidin that stains actin and are shown at identical magnification. UB, ureteric bud. Bar = 100 μm. B: analysis of the number of extensions/tubules per ureteric bud cell cyst after treatment with 50 μM PD09059 for 0–8 days. Tubulogenesis was progressively inhibited as the duration of treatment with PD09059 increased. C: similar results were seen when ureteric bud cell cysts were treated with UO126. For B and C, 25 cysts for each condition were randomly chosen, and resultant measurements were used to determine the statistical mean and SD. Comparison of means was performed with Student’s t-test (Excel Software package, Microsoft).

Fig. 6. Ureteric bud cell cysts spontaneously initiate tubulogenesis, and tubulogenesis can be inhibited by inhibitors of ERK activation.

We report here two principal findings, both of which are quite interesting. First, we show that MDCK strain I cell cysts, which are likely of collecting duct and hence ureteric bud origin (31), spontaneously initiate tubulogenesis due to higher levels of active ERK. MDCK strain II cells, which have lower levels of active ERK (17), do not spontaneously tubulate. Active ERK is both necessary, as ERK inhibitors prevent the initiation of tubulogenesis, and sufficient to initiate tubulogenesis in MDCK strain I cell cysts. Analysis of time-lapse microscopy demonstrates that initiation of tubulogenesis is a highly dynamic process, with cellular extensions forming and retracting rapidly and continuously. Second, we demonstrate that ureteric bud cells also have high levels of active ERK, ureteric bud cell cysts spontaneously initiate tubulogenesis, and tubulogenesis can be inhibited by inhibitors of ERK activation.

An interesting question that we were left with is why only extensions, and not fully formed tubules, spontaneously developed in MDCK strain I cell cysts. It was recently shown that tubulogenesis occurs by a two-step process. ERK is involved in the first stage of tubulogenesis, p-EMT, which occurs during the first 24 h after HGF induction, and MMPs are involved in the second stage, redifferentiation, which occurs 24–72 h after HGF induction (26). In inducible Raf-1 (an indirect activator of ERK) MDCK strain II cells, for fully formed tubules containing lumens to develop during the redifferentiation phase, active ERK must be downregulated (26). Thus the high levels of active ERK may explain why strain I cell cysts predominantly elaborated extensions and rarely, if ever, formed mature tubules.

Branching morphogenesis of tubular epithelium is a common and important feature of vertebrate organogenesis; examples include collecting ducts of the kidney, the airways of the lung, and milk ducts of the mammary gland (6). The MAPK pathway, which is downstream of receptor tyrosine kinases, leads to the phosphorylation, and hence activation, of ERK and has been shown to be important in branching morphogenesis in several systems. For example, the MAPK pathway is essential for branching of the Drosophila tracheal system following binding of the fibroblast growth factor-like molecule Branchless to its receptor tyrosine kinase Breathless (7). Similarly, in murine salivary glands, activated ERK mediates the response to epidermal growth factor and is essential for tubulogenesis (11). Finally, in MDCK II cells grown in a collagen matrix and stimulated with HGF, which acts through the tyrosine kinase c-Met, it was shown that active ERK is necessary and sufficient for tubulogenesis (12, 26). In all of these cases, branching morphogenesis has been shown to be induced by growth factors, produced by the surrounding mesenchyme, that bind to receptor tyrosine kinases.

In the metanephric kidney, development begins at embryonic (E) day 35–37 in humans and E11 in mice, with the appearance of a small epithelial bud or diverticulum from the lower end of the mesonephric (also known as Wolffian) duct near its entry into the cloaca. The metanephros forms as a result of a reciprocal epithelial-mesenchymal induction by-
 tween the ureteric bud and the metanephric mesenchyme (15). It has been shown that ERK regulates branching morphogenesis in the ureteric bud and that the MEK inhibitor PD9059 reversibly inhibits branching in a dose-dependent manner (6).

Although it can be difficult to extrapolate what is occurring in vivo from in vitro data, our results could suggest a model in which a seminal event in the initiation of metanephrogenesis is activation of ERK in the portion of the mesonephric duct/ureteric bud that is destined to form the collecting tubules. How might ERK become activated? It is known that growth factors such as glial cell line-derived neurotrophic factor (GDNF), produced by the metanephric mesenchyme, signal the ureteric bud to branch by binding to the c-ret receptor tyrosine kinase (24, 29, 35). Given that c-ret is expressed in the mesonephric duct before outgrowth of the ureteric bud (37), it is quite possible that GDNF could bind to Ret in just a small portion of the mesonephric duct, activating ERK and allowing metanephrogenesis to occur. It was recently shown that expression of the MAPK pathway inhibitor sprouty is also involved in ureteric bud formation by modulating GDNF/Ret signaling in the Wolffian duct, ensuring that kidney induction is restricted to a single site (4).

Active ERK is necessary and sufficient for the initiation of tubulogenesis in both MDCK strain II cell cysts (26) and, as we show in this study, in MDCK strain I cell cysts. Analysis of the time-lapse microscopy demonstrates that the initiation of tubulogenesis begins with cellular extensions forming and retracting rapidly in a seemingly random fashion. This is reminiscent of what occurs during neurite growth cone formation, where random extensions are stabilized or degraded, depending on the positive and negative factors that they encounter (41). The idea that ERK activation is the seminal event in ureteric bud formation is appealing in that ERK activation also allows for cell proliferation and growth of the ureteric bud. Indeed, ERK was first characterized in the context of proliferative responses of cells to mitogens (5), and when ERK activation was prevented in the ureteric bud, significantly less cell proliferation occurred (6). This proliferative response to activated ERK is consistent with what we found, in that MDCK I cells, with high levels of activated ERK, formed cysts more rapidly than did MDCK II cells.

In summary, the MDCK model, with its two related strains, is uniquely versatile, allowing for a reductionist approach for the study of tubulogenesis. Using an in vitro tubulogenesis assay, we have demonstrated that active ERK is necessary and sufficient for the initiation of tubulogenesis in MDCK strain I cell cysts, as it is for PD90594-induced cysts (26), and that ureteric bud cells also have high levels of active ERK, spontaneously tubulate in a collagen gel, and this tubulogenesis can be inhibited by inhibitors of ERK activation. These data suggest a possible model for the initiation of metanephrogenesis in vivo. It is also likely that ERK is involved in other organ systems that undergo branching morphogenesis, such as the lung, salivary, and mammary glands.

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