Cilia movement regulates expression of the Raf-1 kinase inhibitor protein

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Sas KM, Janech MG, Favre E, Arthur JM, Bell PD. Cilia movement regulates expression of the Raf-1 kinase inhibitor protein. Am J Physiol Renal Physiol 300: F1163–F1170, 2011.—Renal epithelial cell primary cilia act as mechanosensors in response to changes in luminal fluid flow. To determine the role of cilia bending in the mechanosensory function of cilia, we performed proteomic analysis of collecting duct cell lines with or without cilia that were kept stationary or rotated to stimulate cilia bending. Expression of the Raf-1 kinase inhibitor protein (RKIP), an inhibitor of the MAPK pathway, was significantly elevated in rotated cilia (+) cells. This was compared with RKIP levels in cilia (−) cells that were stationary or rotated as well as in cilia (+) cells that were stationary. This result was confirmed in cilia knockout adult mice that had lower renal RKIP levels compared with adult mice with cilia. Downstream of RKIP, expression of phosphorylated ERK was decreased only in cells that had cilia and were subjected to constant cilia bending. Furthermore, elevated RKIP levels were associated with reduced cell proliferation. Blockade of PKC abrogated ciliary bending-induced increases in RKIP. In summary, we found that ciliary movement may help control the expression of the Raf-1 kinase inhibitor protein and thus maintain cell differentiation. In terms of polycystic kidney disease, loss of cilia and therefore sensitivity to flow may lead to reduced RKIP levels, activation of the MAPK pathway, and contribute to the formation of cysts.

polycystic kidney disease; renal epithelial cells; renal cysts

PRIMARY CILIA ARE NONMOTILE projections from polarized, highly differentiated epithelial cells (31). In the kidney, primary cilia are present on nearly all epithelial cells and extend from the apical surface into the tubular lumen, where they respond to luminal fluid flow. Numerous studies have demonstrated that cilia bending with changes in apical flow leads to various intracellular signaling events including increases in intracellular calcium concentrations, thereby acting as mechanosensors (20, 23, 30, 32, 36, 38, 40). Recent studies suggest that primary cilia also serve an important role in the maintenance of cell differentiation (3).

Polycystic kidney disease (PKD) is a ciliopathic disorder associated with increased rates of cell proliferation and apoptosis (17), aberrant cellular polarity (29), and abnormalities in protein trafficking (7). The initiation and progression of PKD has been partially attributed to activation of the MAPK pathway (25, 47, 48). This pathway regulates cell proliferation, apoptosis, and protein synthesis, all of which are altered in PKD (22). In models of PKD, it has been proposed that the altered structure/function of cilia may cause activation of various pathways including the MAPK pathway, leading to cell dedifferentiation and proliferation as well as the other phenotypic characteristics of cystic cells.

Since cilia are unable to synthesize proteins, proteins must be able to travel into and out of the cilium for the formation and proper function of cilia (6). Protein transport occurs in the cilium by intraflagellar transport (IFT). Disruption of this system by mutating genes such as ifi88 (formally called Tg737), which encodes for the IFT protein polaris, results in cilia defects including loss of or severely stunted cilia (53, 54). The Oak Ridge Polycystic Kidney (orpk) mouse is a hypomorph of the ifi88 gene and has severe renal cystic disease as well as other developmental abnormalities. A SV40-temperature-sensitive immortalized orpk collecting duct cell line lacking cilia was developed from this mouse model, along with a cell line in which the wild-type ifi88 gene was reinserted to create a cell line that had cilia (54).

In the present study, we used orpk-derived collecting duct cilia (+) and cilia (−) cell lines to identify differences in proteomic profiles between these two cell lines when kept stationary or subjected to constant rotation to induce cilia bending. To our knowledge, proteomic analyses in response to flow-induced bending of cilia have not been previously performed. Proteins were identified with altered levels of expression between cilia (+) and cilia (−) cells and between stationary and rotated cells. Of note, we found that the levels of expression of the Raf-1 kinase inhibitor protein (RKIP) appeared to be affected by ciliary motion. This was of interest as RKIP is an inhibitor of MAPK signaling and has been shown to play a major role in the pathogenesis of certain forms of cancer. This prompted us to perform additional in vitro and in vivo studies to more closely examine the association between RKIP and cilia.

MATERIALS AND METHODS

Cell culture. Collecting duct cells derived from the orpk mouse model expressing a hypomorphic allele of the Tg737 gene [pcDNA cells, cilia (−)] and genetically rescued cells with the wild-type orpkTg737 gene [BAP2 cells, cilia (+)] were generously donated by Dr. Bradley Yoder (University of Alabama at Birmingham; UAB) (54). Cells were handled identically and grown to confluence in DMEM/F12 media with 0.2 μg/ml dexamethasone, 10 nM triiodothyronine, 1× insulin-transferrin-sodium selenite, 12 U/ml IFN-γ, 268 μg/ml G418, 1% penicillin-streptomycin, and 5% FBS at 33°C, 5% CO2. When confluent, cells were placed at 37°C. 5% CO2 in complete media without IFN-γ or G418 for 5 days until differentiated. All media additives were from Sigma (St. Louis, MO) except for FBS (Thermo Scientific, Waltham, MA). Media was from Mediatech (Manassas, VA). For generation of cilia movement, differentiated cells were subjected to shear stress on an orbital rotator at 1 Hz (33).

Ifi88 mouse. Development of the ifi88 (originally referred to as the Tg737 mouse) floxed allele mouse has been reported in a previous study (16). This mouse line was transferred from UAB to the Medical University of South Carolina (MUSC) where this work was performed. The ifi88 conditional mutant allele was generated.
such that exons 4–6 would be deleted upon Cre recombinase-mediated excision, resulting in a null allele (see Refs. 10 and 16 for a complete description of this mouse). All mice were maintained in accordance with Institutional Animal Care and Use Committee regulations at the MUSC. Genotyping of mice was performed as previously described (16).

For induction of Cre activity, tamoxifen administration was performed once daily for 5 consecutive days when mice were between 8 and 12 wk of age. Tamoxifen (Sigma) dissolved in corn oil (Sigma) was administered (0.5 ml of 10 mg/ml tamoxifen ip). Mice were euthanized 3 wk after tamoxifen injection by isoflurane overdose followed by aortic transection. Kidney tissue was removed and snap-frozen in liquid nitrogen or preserved in 10% buffered formalin.

Two-dimensional gel electrophoresis. Cilia (+) and cilia (−) cells were maintained as described and were incubated in a stationary state or rotated at 1 Hz for 12 h. Cells lysates were collected in a two-dimensional gel electrophoresis (2DE)-compatible buffer [7 M urea, 2 M thiourea, 4% 3-(3-cholamidopropyl)-dimethylammonio)-1-propanesulfonate (CHAPS)] and lysed by tip sonication for 5 min on ice. Lysates were vortexed for 20 min at room temperature and centrifuged at 13,000 g for 10 min at 4°C. The supernatant was removed, and protein concentration was determined by Bradford assay. Rehydration buffer [7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 0.2% bioylates pH 3–10 (Bio-Rad, Hercules, CA)] was added to 125 μg protein to a volume of 200 μl. Proteins were focused using immobilized pH gradient strips (IPG 4–7, Bio-Rad) in a Protean IEF cell (Bio-Rad) for 35,000 V-h with a maximum voltage of 8,000 V and a maximum current of 50 μA/strip. A second dimension separation by SDS-PAGE utilized 11 cm, precast, 4–12% Bis acrylamide and a maximum current of 50 ppm. Peptide mass fingerprinting was used to confirm identifications that were statistically different between stationary and rotated groups using Student’s t-test or one-way ANOVA with Tukey’s post hoc test, as appropriate, using GraphPad Prism (La Jolla, CA). Differences between means were considered significant at values of P < 0.05.

RESULTS

Proteomic identification of differentially expressed proteins following cilia bending. Forty-eight protein spots were differentially abundant between the four groups of cells (q < 0.05), and 46 were identified by tandem mass spectrometry. Proteins that were statistically different between stationary and rotated cilia (+) and cilia (−) cells are listed in Supplemental Table S1 (supplemental material for this article is available online at the journal web site). Proteins whose expression changed in either stationary or rotated cells are listed in Table 1. Phosphatidyethanolamine binding protein 1 (RKIP) abundance was not different between stationary cilia (+) and cilia (−) cells; however, RKIP was elevated 1.30 ± 0.04-fold in cilia (+) cells that were rotated compared with stationary cilia (+) cells. RKIP abundance did not change in cilia (−) cells when shear stress was applied. Only RKIP exhibited this pattern of differential abundance. All identified proteins were analyzed using Ingenuity Pathway Analysis software to determine canonical pathways represented by the differentially abundant proteins. Several pathways were altered in rotated cilia (−) cells that are involved in cell proliferation (Fig. 1).

Cilia bending increases levels of RKIP. To verify the results of the proteomic experiments, differentiated cilia (+) and cilia (−)
Cilia movement regulates RKIP expression

### Table 1. Differentially abundant proteins in stationary or rotated cells by 2-dimensional gel analysis

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Fold-change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary Cilia (+) vs. Cilia (−)</td>
<td></td>
</tr>
<tr>
<td>Enolase</td>
<td>1.3</td>
</tr>
<tr>
<td>Heat shock protein 65</td>
<td>1.2</td>
</tr>
<tr>
<td>Cytosolic malate dehydrogenase</td>
<td>1.2</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein H1</td>
<td>1.2</td>
</tr>
<tr>
<td>Tropomyosin 3-γ</td>
<td>−1.3</td>
</tr>
<tr>
<td>γ-Actin</td>
<td>−1.2</td>
</tr>
<tr>
<td>14-3-3-ζ</td>
<td>−1.1</td>
</tr>
</tbody>
</table>

Shown are proteins identified by 2-dimensional gel analysis as being significantly different in either stationary or rotated cells when protein abundance in cilia (+) is compared with cilia (−) cells. Fold-change indicates whether protein level is higher or lower in cilia (+) cells.

(−) cells were rotated at 1 Hz to stimulate cilia bending or placed in a stationary state for 2 or 12 h. Western blot analysis revealed that RKIP levels were statistically higher in cilia (+) cells rotated for 12 h (1.51 ± 0.18), but not for 2 h (1.00 ± 0.08) (Fig. 2). RKIP levels in cilia (−) cells did not differ significantly from stationary cilia (+) cells at 12 h (1.02 ± 0.03 stationary, 1.05 ± 0.12 rotating) or at 2 h (1.26 ± 0.19 stationary, 1.06 ± 0.11 rotating). These results confirmed that RKIP levels are increased following bending of cilia.

Cilia bending correlates with reduced levels of pERK. Differentiated cilia (+) and cilia (−) cells were rotated at 1 Hz to stimulate cilia bending or placed in a stationary state for 12 h. Western blot analysis revealed that pERK levels were lower in rotated cilia (+) cells compared with stationary cilia (+) cells (0.50 ± 0.07), whereas pERK levels in cilia (−) cells were not significantly different from stationary cilia (+) cells (0.90 ± 0.42 stationary, 0.95 ± 0.28 rotating) (Fig. 3). This finding suggests that the increased levels of RKIP function to suppress signaling through the MAPK pathway.

Cellular proliferation rates and cilia bending. Differentiated cilia (+) and cilia (−) cells were rotated at 1 Hz to stimulate cilia bending or placed in a stationary state for 0, 12, 24, or 48 h, and cell proliferation was determined at each time point by measuring the total amount of DNA. When the proliferation of rotated cells compared with stationary cells was examined, rotated cilia (−) cells had higher proliferation rates of proliferation at 12 (1.41 ± 0.19) and 24 h (1.27 ± 0.09) while cilia (+) cells had similar rates between conditions (0.97 ± 0.05 12 h, 0.99 ± 0.04 24 h) (Fig. 4), suggesting that ciliary movement helps suppress proliferation. This finding also supports the suggestion that enhaced RKIP expression upon cilia bending helps to suppress signaling through the MAPK pathway, one of the primary pathways controlling cellular proliferation.

RKIP levels are significantly reduced in mice lacking cilia. Mice with a conditional floxed allele to the iff88 gene were given tamoxifen to knockout the IFT protein polaris. After 3 wk, >80% of polaris and cilia were absent from epithelial cells in the kidney (16). RKIP levels from mouse kidney tissue lysates were determined by Western blot analysis. Mice in which cilia had been depleted expressed significantly lower levels of RKIP (0.23 ± 0.05) than mice with cilia (0.54 ± 0.04) (Fig. 5). Additionally, staining for RKIP in mouse kidney sections identified that not only are RKIP levels significantly lower in cilia (−) mice but also RKIP staining is most predominant in proximal convoluted tubules of cilia (+) mice (Fig. 6). These findings support the in vitro results, suggesting that cilia expression in vivo does correlate with RKIP levels.

RKIP levels with cilia bending are diminished by a PKC inhibitor. Differentiated cilia (+) and cilia (−) cells were rotated at 1 Hz to stimulate cilia bending or placed in a stationary state for 12 h following the addition of inhibitors for PKA, PKC, or the calcium chelator BAPTA. Western blot analysis revealed that the inhibitors had no significant effect on RKIP levels in cells without cilia; however, the PKC inhibitor significantly reduced the levels of RKIP in rotated cilia (+) cells (Fig. 7). The reduction of RKIP back to near baseline levels in cilia (+) cells suggests that PKC has a role in RKIP regulation in response to cilia bending.

**DISCUSSION**

Primary cilia at the apical membrane of renal epithelial cells act as mechanosensors by sensing the external environment and transmitting this information into the cells (31). One consequence of this information transfer is to alter the expression pattern and function of cellular proteins. A goal of this study was to identify differentially expressed proteins in stationary kidney collecting duct cell lines with or without cilia and then compare this to the pattern of protein expression in rotated cells, which would induce cilia bending. One protein that was identified that had significantly elevated expression in cilia (+) cells upon cilia bending: RKIP.

The MAPK signaling pathway involves signaling through the Ras/Raf/MEK/ERK cascade. This pathway is important for various cellular functions including growth and differentiation.
Since this pathway is critical for a number of cellular processes, its expression can be activated/repressed by numerous receptors and control of this pathway can occur at multiple points, especially at the level of Raf-1 (22). RKIP has been found to inhibit Raf-1 activity and therefore keep the MAPK pathway in check (46, 51). RKIP also inhibits transcription of NFkB (44, 52) as well as inhibiting G protein-coupled receptor (GPCR) signaling by regulating G protein-coupled receptor kinase-2 (GRK2) (21). Since RKIP is a regulator of intracellular signaling pathways, especially inhibition of Raf-1 kinase, this protein has been studied in cancer metastasis. Reduced RKIP levels have been linked to human metastatic tumors from prostate (13, 18), breast (8, 15), colorectal (2), and skin (37), while RKIP overexpression in prostate (12, 13), liver (19), and skin (37) metastatic cell lines resulted in decreased tumor invasiveness. Additionally, RKIP has been shown to regulate the mitotic spindle checkpoint and has also been linked to chromosomal instabilities (1, 11, 46). The fact that RKIP expression appears to be affected by ciliary bending is intriguing in light of the fact that PKD is considered to be a ciliopathic disease. This prompted us to further assess the role of cilia bending in controlling RKIP expression.

To specifically examine RKIP expression, collecting duct cilia (+) and cilia (−) cells were grown stationary or were rotated to stimulate cilia bending. Western blot analysis of cellular lysate revealed that RKIP expression was the same in cilia (+) and cilia (−) cells that were stationary or rotated for 12 h. Cells were incubated stationary (S) or rotated (R) at 1 Hz to stimulate cilia bending. RKIP levels were normalized to GAPDH, and relative band density is presented compared with RKIP levels in stationary cilia (+) cells. *P < 0.05; n = 5.

Fig. 2. Western blot analysis for Raf-1 kinase inhibitor protein (RKIP) in cilia (+) and cilia (−) cells at 12 (A) or 2 h (B). Cells were incubated stationary (S) or rotated (R) at 1 Hz to stimulate cilia bending. RKIP levels were normalized to GAPDH, and relative band density is presented compared with RKIP levels in stationary cilia (+) cells. *P < 0.05; n = 5.

Since this pathway is critical for a number of cellular processes, its expression can be activated/repressed by numerous receptors and control of this pathway can occur at multiple points, especially at the level of Raf-1 (22). RKIP has been found to inhibit Raf-1 activity and therefore keep the MAPK pathway in check (46, 51). RKIP also inhibits transcription of NF-kB (44, 52) as well as inhibiting G protein-coupled receptor (GPCR) signaling by regulating G protein-coupled receptor kinase-2 (GRK2) (21). Since RKIP is a regulator of intracellular signaling pathways, especially inhibition of Raf-1 kinase, this protein has been studied in cancer metastasis. Reduced RKIP levels have been linked to human metastatic tumors from prostate (13, 18), breast (8, 15), colorectal (2), and skin (37), while RKIP overexpression in prostate (12, 13), liver (19), and skin (37) metastatic cell lines resulted in decreased tumor invasiveness. Additionally, RKIP has been shown to regulate the mitotic spindle checkpoint and has also been linked to chromosomal instabilities (1, 11, 46). The fact that RKIP expression appears to be affected by ciliary bending is intriguing in light of the fact that PKD is considered to be a ciliopathic disease. This prompted us to further assess the role of cilia bending in controlling RKIP expression.

To specifically examine RKIP expression, collecting duct cilia (+) and cilia (−) cells were grown stationary or were rotated to stimulate cilia bending. Due to the rotation method used, it is possible that all cells were not exposed to the same amount of shear stress; however, the centripetal force generated by rotation was satisfactory for cilia bending. Western blot analysis of cellular lysate revealed that RKIP expression was the same in cilia (+) and cilia (−) cells that were stationary or rotated for 12 h. Cells were incubated stationary (S) or rotated (R) at 1 Hz to stimulate cilia bending. RKIP levels were normalized to GAPDH, and relative band density is presented compared with RKIP levels in stationary cilia (+) cells. *P < 0.05; n = 5.

Fig. 3. Western blot analysis for phosphorylated ERK (pERK) in cilia (+) and cilia (−) cells at 12 h. Cells were incubated stationary (S) or rotated (R) at 1 Hz to stimulate cilia bending. pERK levels were compared with total ERK levels, and relative band density is presented compared with stationary cilia (+) cells. Although not significant by 1-way ANOVA, there was significance between stationary and rotating conditions in cilia (+) cells when examined by t-test; n = 5.

Fig. 4. Cellular proliferation as determined by DNA content for cilia (+) and cilia (−) cells. Cells were incubated stationary or rotated at 1 Hz to stimulate cilia bending for 0, 12, 24, or 48 h. DNA content was determined and is presented as amount in rotated cells compared with amount in stationary cells at each time point. *P < 0.05 between cilia (+) and cilia (−); n = 3 (0 h) and n = 4 (12, 24, and 48 h).
and was similar to cilia (−) cells that were rotated. Only in cilia (+) cells subjected to rotation to stimulate cilia bending was there an elevation in RKIP levels. This elevation was seen after 12 h of rotation but not after 2 h. This suggests that cilia bending may be inducing RKIP synthesis and is consistent with the data obtained by proteomic analysis. To determine whether there were actual differences in downstream MAPK signaling, we measured pERK abundance in cilia (+) and cilia (−) cells with and without rotation. pERK was only suppressed in cilia (+) cells that were rotated, suggesting that this was the result of the elevated RKIP levels.

Additional studies were performed to measure cell proliferation in stationary and rotated cilia (+) and cilia (−) cells. We found that rotated cilia (−) cells had elevated rates of cell proliferation compared with stationary cells. This was also seen in the proteomic experiments, as pathways involved in cell proliferation were altered in rotating cilia (−) cells (Fig. 1) and levels of proliferating cell nuclear antigen were increased in rotated cilia (−) cells compared with all other groups (Supplemental Table S1). It is possible that rotation would lead to enhanced mixing of cell culture media and would increase the availability of nutrients and oxygen to growing epithelial cells. Presumably, this same effect should have occurred in the cilia (+) cells. Regardless, the difference in proliferation rates between rotated cilia (−) cells and cilia (−) cells supports the finding of RKIP upregulation in rotated cilia (+) cells and the idea that RKIP may be functionally important in limiting proliferation. Unfortunately, there is a lack of pharmacological tools that specifically inhibit RKIP. In addition, there are at least five RKIP-like sequences in the mouse genome, with data on renal expression being scarce (45). As small-molecule drug design and use were beyond the scope of this study, we were unable to verify that the changes in the rate of cell proliferation were unequivocally due to the changes in RKIP expression. However, RKIP helps regulate the mitotic spindle checkpoint, and loss of RKIP has been shown to increase the rate at which cells progress through mitosis (11). Therefore, an increase in RKIP should control the rate of cell proliferation, such as seen here.

RKIP acts to reduce the MAPK signaling response to stimuli such as EGF (14, 46). This is of interest in PKD research since cystic fluid has been shown to contain a higher concentration of EGF receptor (EGFR) ligands (35, 42, 50, 55). In addition, EGFR mislocalizes to the apical membrane in several models of PKD so that EGFRs are exposed to cystic fluid and thus high levels of EGFR ligands (26, 34). Therefore, RKIP diminution in cystic cells may be even more severe as EGF signaling is increased, leading to increased MAPK signaling. Additionally, RKIP has been shown to regulate B-Raf activity in addition to Raf-1 kinase activity (28). This is interesting as cAMP activation of B-Raf is believed to be one of the main mechanisms of ERK activation in PKD (27, 47, 49). It is important to note that our studies do not provide a direct link between RKIP and cystogenesis. We present the important finding that RKIP is regulated by cilia.

To confirm that RKIP levels are regulated by cilia in vivo, mice containing a conditional floxed allele for the ift88 gene were used to generate cilia (+) and cilia (−) mice. RKIP levels were measured 3 wk after tamoxifen treatment, which is well before the presence of discernable cysts in the cilia (−) mice. RKIP levels were significantly decreased in the kidneys of cilia (+) mice.
mice, confirming that cilia are important for RKIP expression. When examining RKIP expression in the kidney by immunohistochemical staining, it appeared that RKIP levels were highest in the proximal tubules and appeared to be lower in distal tubular segments in the cilia kidneys. Cysts predominately form in the collecting ducts or distal tubules (27, 29, 39, 43), leading us to speculate that perhaps the lower levels of RKIP in collecting duct cells make this segment more susceptible to cystic formation.

To examine potential regulators of RKIP expression in cilia (+) and cilia (−) cells, we utilized inhibitors of PKA and PKC, as well as the calcium chelator BAPTA. RKIP expression was decreased upon cilia bending with the PKC inhibitor, suggesting that PKC is involved in the regulation of RKIP expression by cilia. This was an interesting finding since PKC is known to phosphorylate RKIP, leading to RKIP dissociation from Raf-1 and subsequent inhibition of GRK2 (9, 21). Although PKC is known to play a role in the modulation of RKIP function (MAPK vs. GPCR), it is somewhat surprising that it may play a role in RKIP expression. One scenario is that cilium bending increases cytosolic calcium concentrations, leading to the activation of a calcium-sensitive isoform of PKC, which then stimulates RKIP expression. In this regard, the calcium chelator BAPTA tended to decrease RKIP levels, although not significantly. However, it is very difficult to know how effectively BAPTA is influencing cell calcium levels over the course of hours. The expression of RKIP in metastatic cell lines is repressed by the zinc finger transcription factor SNAIL, which is also known to modulate epithelial-mesenchymal transition (5). SNAIL expression is reduced upon inhibition of NF-κB (4), suggesting that NF-κB activation leads to the induction of SNAIL and reduction of RKIP. Whether a similar relationship among NF-κB, SNAIL, and RKIP exists in cilia (−) cells as well as whether PKC inactivation leads to changes in SNAIL expression in cilia (+) cells remains to be investigated.

Although this is the first known work examining proteomic alterations following cilium bending, widespread gene expression analysis has been performed in renal cystic tissues (41). While several genes involved in MAPK signaling were upregulated (including Raf-1 and ERK1), RKIP gene expression was downregulated 2.4-fold. This downregulation of gene expression in tissue derived from human autosomal dominant PKD cysts supports our findings of decreased RKIP protein expression in cells that do not have cilia. We believe therefore that bending of the primary cilium leads to enhanced RKIP expression, potentially downstream of PKC activation, which then diminishes MAPK signaling and helps to control cellular proliferation (Fig. 8). When the cilia are lost or function is inhibited, as in PKD, RKIP expression is decreased, leading to enhanced MAPK signaling and cellular proliferation.

In summary, we have identified through proteomic analysis that expression of the Raf-1 kinase inhibitor protein is significantly increased upon cilia bending, and this correlates with decreased levels of pERK and therefore inhibition of the MAPK pathway. Cilia movement may modify RKIP levels through the activation of PKC. These findings suggest that it may not be just the presence of cilia but rather cilary movement that is essential for the maintenance of cell differentiation and suppression of cell proliferation and that this occurs, at least in part, through regulation of the Raf-1 kinase inhibitor protein.

Fig. 7. Western blot analysis for RKIP in cilia (+) and cilia (−) cells following incubation with inhibitors for PKA, PKC, or a calcium chelator for 12 h. Cells were incubated while stationary (S) or rotated (R) at 1 Hz immediately after addition of treatment. RKIP levels were normalized to GAPDH, and relative band density is presented as amount in rotated cells compared with amount in stationary cells. *P < 0.05; n = 3.

Fig. 8. Schematic of proposed signaling pathway in normal kidney cells. Cilium bending in response to tubular flow allows for calcium entry into the cell. This results in the translocation of PKC to the cell membrane, where it leads to an increase in RKIP. RKIP then suppresses signaling through the MAPK/ERK pathway, leading to control of cellular proliferation and maintenance of cell differentiation.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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CILIA MOVEMENT REGULATES RKIP EXPRESSION


