A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia

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The cilium is a microtubule-based organelle that contains a unique complement of proteins for cell motility and signalling functions. Entry into the ciliary compartment is proposed to be regulated at the base of the cilium1. Recent work demonstrated that components of the nuclear import machinery, including the Ran GTPase and importins, regulate ciliary entry2-4. We hypothesized that the ciliary base contains a ciliary pore complex whose molecular nature and selective mechanism are similar to those of the nuclear pore complex. By microinjecting fluorescently labelled dextrans and recombinant proteins of various sizes, we characterize a size-dependent diffusion barrier for the entry of cytoplasmic molecules into primary cilia in mammalian cells. We demonstrate that nucleoporins localize to the base of primary and motile cilia and that microinjection of nucleoporin-function-blocking reagents blocks the ciliary entry of kinesin-2 KIF17 motors. Together, this work demonstrates that the physical and molecular nature of the ciliary pore complex is similar to that of the nuclear pore complex, and further extends functional parallels between nuclear and ciliary import.

Cilia are evolutionarily conserved organelles important for normal cellular development, motility and sensory functions5,6. Disruption of proteins that normally localize to and function within primary cilia leads to a wide range of human diseases collectively termed the ciliopathies, with phenotypes including retinal degeneration, cystic kidney diseases, skeletal defects and obesity7-9. As organelles with a unique protein and lipid composition, cilia must use specific mechanisms to ensure the accurate targeting to, and retention of proteins within, this compartment10.

The mechanisms regulating entry into the ciliary compartment are unclear at first glance, as the cilium lacks a limiting membrane separating it from the cytoplasm. The restriction point is believed to be a specialized region at the base of the cilium called the transition zone, where Y-shaped structures and transition fibres can be seen by electron microscopy to link the microtubule core (the axoneme) and basal body structures to the ciliary membrane11-13. Recent work has uncovered a network of protein components that localize to the transition zone and basal body region, and whose defects lead to various ciliopathies14-18.

Are cytoplasmic proteins restricted from entering the ciliary compartment, and if so, what mechanisms prevent diffusive entry? We recently demonstrated that ciliary entry of a cytoplasmic protein, the kinesin-2 motor KIF17, requires an import signal similar to a nuclear localization signal (NLS), the nuclear transport factor importin-β2 (transportin-1), and a RanGTP/GDP gradient between ciliary and cytoplasmic compartments2. Thus, we hypothesized that the mechanisms that regulate ciliary entry of soluble proteins may be mechanistically similar to those that regulate nuclear entry. Consistent with this hypothesis, ciliary import of X-linked retinitis pigmentosa protein RP2 uses importin-β2 and NLS-like sequences4. In addition, the transition-zone region has been compared to the nuclear pore complex (NPC) and proposed to serve as a flagellar/ciliary pore complex (CPC; refs 1,10). Thus, we set out to determine whether primary cilia use mechanisms similar to those of nuclei to regulate the entry of cytoplasmic components.

Nuclear–cytoplasmic shuttling is controlled by the NPC, a large multiprotein complex embedded in the double membrane of the nuclear envelope to form a pore-like structure19. Transport of cytoplasmic components through NPCs is regulated in two ways20,21. First, a size-exclusion mechanism limits molecules of relative molecular mass greater than 30,000 (Mr > 30K) from freely diffusing between compartments. Second, proteins above this size limit use NLS import signals, nuclear transport receptors and the small GTPase Ran to cross the physical barrier formed by the NPC.

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We first tested whether there is a size-dependent barrier that restricts ciliary entry of cytoplasmic molecules by microinjecting ciliated mammalian cells with fluorescently labelled dextrans of various sizes, analogous to previous work defining a diffusion barrier for the NPC (refs 22,23). hTERT-RPE cells were transfected with mCherry-tagged Arl13b (ADP-ribosylation factor-like 13b) as a live-cell marker of primary cilia (Supplementary Fig. S1) and observed 48 h later by wide-field fluorescence microscopy. Only cells with Arl13b–mCherry-labelled cilia that projected off the cell body were microinjected with fluorescent dextrans to clearly distinguish cilia from cytoplasmic fluorescence. Dextran localization to the nuclear and ciliary compartments was assessed 20 min after microinjection. Small dextrans (Mr 3K and Mr 10K) freely diffused into both ciliary and nuclear compartments (Fig. 1a,b). In contrast, larger dextrans (Mr 40K and Mr 70K) were excluded from both ciliary and nuclear compartments (Fig. 1c,d). Quantification of the fluorescence intensities in primary cilia demonstrates that the Mr 40K and Mr 70K dextrans were significantly restricted from entering the cillum (Fig. 1e). Ciliary entry of Mr 3K and Mr 10K dextrans was rapid and could be detected within 5 min post injection (Supplementary Fig. S1c), consistent with the rapid diffusion of small molecules into and within sea urchin spermatozoa24.

To further investigate the size-dependent diffusion barrier for ciliary entry, we microinjected fluorescently labelled soluble proteins of nearly spherical shape that act as inert probes25. Again, only cells with Arl13b–mCherry-labelled cilia projecting off the cell body were selected for microinjection and analysis. We found that α-lactalbumin (Mr 14K), recombinant green fluorescent protein (rGFP, Mr 27K) and protein A (Mr 41K) could enter both ciliary and nuclear compartments (Fig. 2a,b,d,f). Analysis in live cells is a critical component of these experiments as ciliary localization of expressed GFP could be reproducibly seen in live cells that were transfected (Fig. 2c,f) but was difficult to determine after fixation and/or permeabilization (data not shown), consistent with recent reports26,27. In contrast to the ciliary localization of these smaller proteins, bovine serum albumin (BSA, Mr 67K) was restricted from both ciliary and nuclear compartments (Fig. 2e,f). Collectively, these experiments characterize a barrier for diffusion of cytoplasmic molecules into the ciliary compartment. Thus, similarly to nuclei, cilia restrict the diffusional entry of cytoplasmic molecules on the basis of size and use active transport mechanisms to facilitate entry of large proteins.

What are the molecular mechanisms by which ciliary entry of cytoplasmic molecules is restricted? One possibility is that ciliary-specific proteins create a diffusional barrier resembling that of the nuclear pore. Alternatively, NPC components could localize to the ciliary base and create a permeability barrier. To test this latter possibility, we tested whether NPC components localize to primary cilia in mammalian cells. The NPC is a large proteinaceous structure composed of multiple copies of 30 different nucleoporins that assemble into subcomplexes (Fig. 3a; refs 28,29). Enhanced GFP (eGFP)–nucleoporins have been used to study the kinetics and organizational dynamics of the NPC in live cells30,31. We expressed a fluorescently tagged nucleoporin from each subclass in Odora cells, an immortalized cell line derived from rat olfactory sensory neurons that generates primary cilia32. All nucleoporins localized to the nuclear membrane as expected (Supplementary Fig. S2). Localization at the ciliary base was observed for eGFP–NUP37 (outer ring nucleoporin, 12/15 cells; Fig. 3e), eGFP–NUP35 (inner ring nucleoporin, 18/18 cells; Fig. 3f), NUP93–eGFP3 (linker nucleoporin, 19/19 cells; Fig. 3g), and NUP62–eGFP3 (central phenylalanine–glycine (FG) nucleoporin, 16/17 cells; Fig. 3h). Ciliary base localization was also observed when fluorescently tagged nucleoporins were expressed in hTERT-RPE cells (Supplementary Fig. S3). In addition, eGFP–NUP214, a member of the cytoplasmic FG-containing nucleoporin and filament subcomplex, localized in distinct puncta near the γ-tubulin-labelled basal body (Fig. 3c).
Interestingly, not all nucleoporins could be localized at the ciliary base. POM121–3GFP, GFP–GP210 and NDC1–GFP, transmembrane nucleoporins that anchor the NPC to the nuclear envelope, did not localize to the base of the primary cilium (Fig. 3d and Supplementary Fig. S2h,i). Ciliary localization was also not observed for eGFP–NUP153, a nucleoporin that projects into the nucleoplasm (Fig. 3i). These eGFP–nucleoporins localized correctly to the nuclear envelope (Supplementary Fig. S2), suggesting that the GFP tag does not hinder their ability to assemble into nucleoporin complexes. Collectively, these experiments demonstrate that specific nucleoporins localize to the ciliary base, where they could regulate passive and active transport into the ciliary compartment.

To determine if endogenous nucleoporins localize to the base of the cilium, we immunostained cells with a monoclonal antibody, mAb414, that recognizes several FG-containing nucleoporins (NUP358, NUP214, NUP62 and NUP153; ref. 33). The FG repeats on these nucleoporins function to enable cargo–importin complexes to associate with and shuttle through the NPC meshwork. In NIH3T3 cells, mAb414 staining showed discrete puncta present at the base of the cilium, in addition to the nuclear envelope (Fig. 4a). To more precisely define the localization of nucleoporins at the base of cilia, we carried out immunoelectron microscopy of rat trachea. Using antibodies specific to individual nucleoporins, NUP62 and NUP153 could be detected at the ciliary base of epithelial cells (Supplementary Fig. S4c,d).

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To more precisely define the localization of nucleoporins at the base of cilia, we carried out immunoelectron microscopy of rat trachea. Using mAb414 in single-label immunogold electron microscopy, gold particles labelled the ciliary transition zone and basal body in addition to the expected localization at NPCs on the nuclear envelope (Supplementary Fig. S5b,d). In double-label immunogold electron microscopy with mAb414 and antibodies to CEP290 (also known as NPHP6), co-staining was observed in clusters (Fig. 4f) and as single gold particles (Fig. 4g) at the transition zone. Thus, whereas mAb414 staining seems to primarily co-localize with the basal body by fluorescence microscopy, mAb414 antigens localize at the transition zone and basal body by electron microscopy, perhaps owing to differences in antigen accessibility. Further work is required to define the localization of specific nucleoporins at the ciliary base. Collectively, fluorescence and electron microscopy experiments demonstrate that endogenous nucleoporins localize not only in the NPCs of the nuclear envelope but also in CPCs at the ciliary base.

To determine whether nucleoporins function to regulate import of ciliary proteins, we microinjected two different NPC-function-blocking reagents into ciliated cells, mAb414 (ref. 35) and a truncated version of importin-β1 (ref. 36). NIH3T3 cells co-expressing KIF17–mCitrine and Arl13b–mCherry were microinjected with mAb414 or importin-β1 (45–462) together with TAMRA dye to mark injected cells (Fig. 5). As controls, cells were injected with TAMRA dye alone or not injected. KIF17–mCitrine fluorescence at the tips of cilia was photobleached and the subsequent fluorescence recovery (Fig. 5b–e) was taken as a measure of ciliary entry of new KIF17–mCitrine molecules as described. Microinjection of mAb414 or importin-β1 (45–462) resulted in a significant reduction in KIF17–mCitrine fluorescence recovery when compared with control cells (Fig. 5d,e). We conclude

**Figure 2** The ciliary base acts as a size-dependent barrier for entry of inert cytoplasmic proteins. (a–e) HTERT-RPE cells expressing Arl13b–mCherry to mark the primary cilium in live cells were either microinjected with Alexa 448-labelled purified proteins α-lactalbumin (M, 14K) (a), recombinant GFP (rGFP, M, 27K) (b), protein A (M, 41K) (d) or BSA (M, 67K) (e) or were transfected with GFP-expressing plasmid (c). Shown are representative images taken 5 min post injection. White asterisks indicate the nuclei. Dashed white lines indicate the cell periphery. The bottom row shows higher magnifications of primary cilia in the boxed regions. White arrows point to primary cilia. Scale bars, 10 μm. (f) Quantification of ciliary localization of microinjected fluorescent proteins. The data are expressed as a diffusion-barrier index. Error bars represent s.e.m. N = 11 (a), 10 (b), 7 (c), 8 (d), 11 (e) cells.
Figure 3 Fluorescently tagged nucleoporins localize to the base of primary cilia. (a) Diagram depicting the overall structure and subcomplexes of the NPC, based on the model in ref. 37. (b–i) Odora cells expressing eGFP–NUP214 (c), POM121–3GFP (d), eGFP–NUP37 (b,e), eGFP–NUP35 (f), NUP93–eGFP3 (g), NUP62–eGFP3 (h) and eGFP–NUP153 (i) were fixed and stained with antibodies to acetylated α-tubulin (AcTub, red) and γ-tubulin (magenta) to mark the primary cilium and basal bodies, respectively. Merged images are shown in the right panels. Shown are representative images of the ciliary region of the cells; whole-cell views are shown in Supplementary Fig. S2. Scale bars, 5 μm.
Figure 4 Endogenous nucleoporins localize to the base of cilia. (a) Representative image of an NIH3T3 cell fixed and stained with antibodies to nucleoporins (mAb414, green) and acetylated α-tubulin (AcTub, red) to mark the primary cilium. The right panel shows a higher magnification of the primary cilium in the boxed region. (b) Rat trachea cells were fixed and stained to nucleoporins (mAb414, green), acetylated α-tubulin (AcTub, red) and γ-tubulin (γ-Tub, magenta). (c) Rat trachea cells were fixed and stained to nucleoporins (mAb414, green), acetylated α-tubulin (red) and SDCCAG8 (magenta). 4,6-diamidino-2-phenylindole (DAPI, blue) indicates the nucleus. Scale bars, 5 μm. (d) A schematic representation of the epithelial cells in (b,c) in which the red line indicates the confocal section and the black arrow represents the point of view. (e–g) Rat trachea tissue was fixed and processed for transmission electron microscopy. (e) Addition of osmium tetroxide during electron microscopy processing resulted in higher contrast. Scale bar, 500 nm. (f,g) Dual-label immunogold electron microscopy of sections of tracheal tissue using mAb414 and antibodies to CEP290. Scale bars, 100 nm.

that functionally inhibiting nucleoporins restricts KIF17–mCitrine entry into the ciliary compartment.

In conclusion, we propose a model in which ciliary import shows selective and molecular features characteristic of nuclear import. First, we demonstrate that soluble molecules above a specific size threshold are restricted from passively entering the ciliary compartment, in an analogous manner to that in which the NPC acts as a sieve to prevent spurious entry of molecules. This CPC permeability barrier thus enables the enrichment of specific proteins destined for the cilium through active transport mechanisms. Furthermore, we show that several nucleoporins localize to and function at the base of the cilium in a similar manner to their functional roles at the NPC. Nucleoporins are found in several cilia proteomes (http://v3.ciliaproteome.org/cgi-bin/index.php). Thus, we propose that nucleoporins not only form the molecular components that regulate transport across the NPC, but also transport across the CPC. Collectively with our previous work2,4, we demonstrate that ciliary import uses the three defining functional components that regulate nucleocytoplasmic transport—nuclear transport factors such as importins, the Ran GTPase system and nucleoporins. Interestingly, not all fluorescent nucleoporins localize to the base of the cilium. The absence of eGFP3–NUP153 at the cilium base may be due to the nuclear-specific functions of this subcomplex, which...
Figure 5 Microinjection of nucleoporin-function-blocking reagents into cells restricts the ciliary entry of KIF17 motors. (a–e) NIH3T3 cells co-expressing KIF17–mCitrine and Arl13b–mCherry were uninjected (a,b) or were injected with fluorescent TAMRA dye alone (c), mAb414 antibodies and TAMRA (d) or importin-β1 (45–462) (Imp45–462) and TAMRA (d). Expressing cells were imaged (a, prebleach) and then the KIF17–mCitrine fluorescence at the distal tip of the cilium was bleached with high laser power. The dashed white line indicates the edge of the cell. Scale bar, 10µm. Following the bleach, the cells were imaged (postbleach) and the fluorescence recovery of KIF17 was measured over time. (b–f) Prebleach, postbleach and recovery images of KIF17–mCitrine in the cilium. Arrowheads point to the distal tip of the cilium. (f) Quantification of the fluorescence recovery of KIF17–mCitrine in the distal tips of cilia. The data are represented as mean ± S.E.M. of fluorescence recovery after photobleach. N = 8 (a), 8 (b), 8 (c) and 7 (d). Statistical significance was assessed by Student’s t-test. n.s., no significant difference.
forms a basket structure that serves as a platform for transcriptional regulation and chromatin stability in the nucleoplasm.\(^7\) NUP153 was demonstrated not to be required for transportin-mediated nuclear import in *Xenopus* nuclei.\(^8\) The absence of POM121–3GFP, GFP–GP210 and NDC1–GFP from the ciliary base may be due to their nuclear-specific roles in anchoring components of the NPC in the nuclear envelope.\(^9\) Indeed, the structural differences between nuclear and ciliary membranes suggest that a different membrane-associated protein complex anchors the CPC to the surrounding ciliary membrane. It is tempting to speculate that the nephropathies (NPHP) and Meckel–Gruber syndrome (MKS) disease-related gene networks may play a role in anchoring cilia near the CPC. Recent work demonstrated that disruption of NPHP and/or MKS genes caused defects in anchoring transition zone structures to the ciliary membrane and abnormal ciliary protein composition.\(^14\)–\(^18\) Clearly, further studies are required to determine whether NPHP, MKS and nucleoporin components are functionally integrated together at the base of the cilium.

As ciliary and nuclear import share several molecular and mechanistic features, how cargo are distinguished from entering the ciliary versus the nucleus is unclear. Cilia have evolved distinct mechanisms for ciliary trafficking that may be important in defining ciliary versus nuclear transport, such as the intraflagellar transport complex that drives transport along axonemal microtubules, the BBSome coat complex, and small GTPases of the Arf and Rab families (reviewed in refs 1,10). Future work is clearly required to uncover the complex molecular network at the base of the cilium and to delineate the mechanisms by which these components establish the primary cilium as a complex signalling centre and their implications in ciliopathies.

**METHODS**

Methods and any associated references are available in the online version of the paper at www.nature.com/naturecellbiology

*Note: Supplementary Information is available on the Nature Cell Biology website*
METHODS

Antibodies and plasmids. Commercial antibodies include acetylated α-tubulin (1:10,000; clone 6-11B-1, Sigma), γ-tubulin (1:500; T6557, Sigma), mAb414 (1:400 for immunofluorescence, 1:50 for immunoelectron microscopy; ab24609, Abcam), NUP62 (1:250; sc-48373, Santa Cruz), polyglutamylated tubulin (1:1,000; GT335, Enzo Life Sciences) and CEP290 (1:50 for immunoelectron microscopy; EHC-00365, Bethyl Laboratories). Rabbit polyclonal anti-acetylated tubulin antibody (1:1,000) was raised against the synthetic peptide CGQMPSD(AcK)TIGGGDD. NUP133 was at 1 mg ml

10% fetal calf serum and 1% penicillin

485-152), DyLight 594-conjugated anti-rabbit (711-515-152) and Texas Red-eGFP

plasmid was constructed by subcloning human Arl13b complementary DNA

fluorescence intensity in the cytoplasmic region half the distance between the nuclear ciliary region of interest. The average fluorescence in the ciliary region of interest

by the Arl13b

injection to remove any aggregates.

Microinjection. Fluorescently labelled dextan of different molecular weights—M, 3K–FITC, M, 10K–FITC, M, 40K–FITC and M, 70K–FITC (Molecular Probes)—were reconstituted in buffer containing 25 mM HEPES at pH 7.4, 115 mM KOAc, 5 mM NaOAc, 5 mM MgCl, 0.5 mM EDTA, 1 mM GTP and 1 mM ATP and microinjected into cells at 10 mgml

1.

HtERT-RPE cells were microinjected and visualized using the FemtoJet Microinjector System (Eppendorf) mounted on an inverted epifluorescence microscope (Nikon TE2000-E) with 40

40 objectives and a Photometrics CoolSnap HQ camera.

Fluorescence recovery after photobleaching. NIH3T3 cells were plated in glass-bottom dishes (MatTek), co-transfected with plasmids encoding KIF17–mCitrine and Arl13b–mCherry, and serum starved for 24–48 h. Expressing cells were microinjected with TAMRA dye alone, TAMRA dye (30 μM) + mAb414 antibody (0.95 mg ml

1), or TAMRA dye (50 μM) + importin-β1 (45–462) (133 μM) using the FemtoJet microinjector system (Eppendorf) mounted onto a Nikon Ti Eclipse inverted microscope. The cells were maintained in a live-cell chamber at 37 °C and 5% CO

2. Using the Nikon A-1 confocal system with Perfect Focus, microinjected cells were identified by the presence of the TAMRA dye. A prebleach picture was taken and then the KIF17–mCitrine fluorescence at the distal tip of cilia was photobleached using 50% laser power for 1 s. KIF17–mCitrine fluorescence recovery images were taken postbleach at 10 min intervals for 30 min. Fluorescence signals were quantified using Metamorph software and background subtracted, and the average fluorescence values from eight cells were plotted using Prism software (GraphPad).

Statistical analysis. All statistical analysis was carried out using Prism software and specific tests are noted in the text. Error bars are ±s.e.m. and significance was assessed as P < 0.05.
Figure S1 Microinjection using Arl13b-mCherry as a live-cell marker for primary cilia. (a) Localization of Arl13b-mCherry to primary cilia in fixed cells. hTERT-RPE cells expressing Arl13b-mCherry (red) were fixed and immunostained for acetylated tubulin (green) to mark the microtubule axoneme of the primary cilium. White asterisks indicate the nuclei. (b, c) Arl13b-mCherry marks the primary cilium in live hTERT-RPE cells used for microinjection. In hTERT-RPE cells, the primary cilium is usually located on the top of the cell, often on top or by the nucleus, which hindered visualization of fluorescent dextrans in the nuclear and ciliary compartments. Thus, cells were chosen for microinjection based on the protrusion of the Arl13b-marked primary cilium off the cell body as shown for the representative cell prior to microinjection in (b). Dashed white lines represent cell periphery. After microinjection of fluorescent dextrans into the cell body, the Arl13b-mCherry (red) and fluorescent dextrans (green) were imaged at various time points. Shown in (c) are images from the same cell in (b) 5 min after microinjection with 3 kDa dextran. White asterisk indicates the nucleus. Far right panels are higher magnifications of the cilium in the white-boxed area. Scale bars, 10 μm.
Figure S2 Fluorescently-tagged nucleoporins localize to the nuclear envelope and, in some cases, to the base of primary cilia in Odora cells. Odora cells expressing (a) EGFP-NUP214, (b) POM121-EGFP3, (c) EGFP-NUP37, (d) EGFP-NUP35, (e) NUP93-EGFP3, (f) NUP62-EGFP3, and (g) EGFP-NUP153, were fixed and stained with antibodies to acetylated α-tubulin (red) and γ-tubulin (magenta) to mark the primary cilium and basal body. Shown are representative confocal sections taken in the plane of the cilium (left panels) and in the cell center (right panels). Odora cells expressing (h) GFP-GP210 and (i) NDC1-GFP were fixed and stained with antibodies to acetylated α-tubulin (red) and γ-tubulin (magenta). GFP-GP210 and NDC1-GFP localize to the nuclear envelope but not the base of primary cilia. Scale bars, 5 μm.
Figure S3 Fluorescently-tagged nucleoporins localize to the base of primary cilia in hTERT-RPE cells. hTERT-RPE cells expressing (a) EGFP-NUP35 or (b) NUP62-EGFP were fixed and stained with antibodies to acetylated α-tubulin or polyglutamylated tubulin (red) to mark the primary cilium. Arrowhead indicates the base of cilia. Scale bar, 5 μm.
**Figure S4** Endogenous nucleoporins localize to the base of motile cilia in rat trachea epithelial cells. Rat trachea cells were fixed and stained with antibodies to nucleoporins (green) using (a, b) mAb414, (c) anti-NUP62 or (d) anti-NUP133. The cells were costained with antibodies to acetylated α-tubulin (red) and SDCCAG8/NPHP10 (magenta). DAPI (blue) indicates the nucleus. Far right images are schematic representations of the epithelial cells in which the red line indicates the confocal section and the black arrow represents the point of view. Ciliary localization of nucleoporins can be visualized in confocal sections (a) along the side of the cell and (b) at base of cilia. Scale bars, 5 μm.
Figure S5 Immunogold EM localization of mAb414 in rat trachea. Rat trachea tissue was fixed and processed for immunogold EM using antibodies to mAb414. (a) Representative TEM image of the tissue surface showing epithelial and goblet cells (scale bar, 5μm). N indicates nuclei of epithelial cells. To the right is a schematic representation of an epithelial cell. Higher magnification TEM views are shown of (b) the cilia base (scale bar, 100 nm), (c) cytoplasm (scale bar, 100 nm), and (d) nucleus (scale bar, 500 nm). Blue arrowheads indicate gold particles to mAb414. The number of gold particles in the cilia base, cytoplasmic, and nuclear regions was counted for 8 cells stained with mAb414 and immunogold secondary antibodies and for 8 cells from a control staining with only immunogold secondary antibodies (no mAb414). Numbers in bold indicate the number of gold particles for mAb414-stained cells; numbers in parentheses indicate the number of gold particles for control cells. Some samples were treated with 4% aqueous uranyl acetate to increase contrast, as shown in (b, right panel) and (c).
Movie S1  Endogenous nucleoporins localize to the base of motile cilia in trachea epithelial cells. Confocal Z-stack of a respiratory epithelial cell immunostained with antibodies to nucleoporins (mAb414, green) and cilia (acetylated a-tubulin, red). DAPI (blue) indicates the nucleus.