

REVIEW

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Nuclear roles for cilia-associated proteins

Tristan D. McClure-Begley and Michael W. Klymkowsky*

Abstract

Cilia appear to be derived, evolutionarily, from structures present in the ancestral (pre-ciliary) eukaryote, such as microtubule-based vesicle trafficking and chromosome segregation systems. Experimental observations suggest that the ciliary gate, the molecular complex that mediates the selective molecular movement between cytoplasmic and ciliary compartments, shares features with nuclear pores. Our hypothesis is that this shared transport machinery is at least partially responsible for the observation that a number of ciliary and ciliogenesis-associated proteins are found within nuclei where they play roles in the regulation of gene expression, DNA repair, and nuclear import and export. Recognizing the potential for such nuclear roles is critical when considering the phenotypic effects that arise from the mutational modification of ciliary proteins.

Introduction

Cilia, whether immotile (primary) or motile, flagella (on sperm), or the cilia-based structures within photoreceptor cells, represent a distinct cytoplasmic and plasma membrane domain [26]. As has been reviewed recently, mutations in cilia- or ciliogenesis-associated proteins can lead to a range of human pathologies, known collectively as ciliopathies [8, 26, 31, 49, 64, 76, 86, 88, 91, 94]. In addition to their well appreciated cilia- and ciliogenesis-associated roles, it is now increasingly apparent that cilia-associated proteins have non-ciliary, cytoplasmic roles, described in a number of recent reviews [87, 93, 102]. For example, it is well established that cilia and cilia-associated proteins are involved in Hedgehog, TGF- β , Wnt, PDGFR α , Notch, and Hippo inter-cellular signaling systems [12, 31, 36, 62, 65, 86]. That said, cilia themselves may not be strictly necessary for a number of these signaling pathways. For example, myeloid and lymphoid cells reportedly lack cilia (primary or motile) yet express cilia-associated proteins [24] and support Hedgehog signaling [16, 93]. Similarly, in the zebrafish, Huang and Schier [37] found that genetic abolition of maternal and zygotic cilia formation left Wnt signaling intact while dysregulating hedgehog signaling.

In addition to their signaling functions, there is clear evidence for the role of a number of cilia-associated proteins in DNA damage repair [4, 102]. In this review, we

highlight the observation that a number of cilia-associated proteins have a nuclear presence, and in some cases, functional nuclear roles. We consider the possibility that the nuclear functions of cilia-associated proteins, arising in part from their evolutionary ancestry, can contribute to ciliopathic phenotypes through both developmentally specific and more generic mechanisms.

Implications of the evolutionary origin of cilia

While speculating about processes that occurred billions of years ago can be problematic, there is a clear consensus that the evolution of cilia began in an ancestor that contained a nucleus, with nuclear pores mediating molecular movement across the nuclear envelope and microtubule-based systems involved in chromosome segregation [10, 27, 58, 59, 70]. Entry into the ciliary domain is regulated through a distinct “transition zone” between non-ciliary cytoplasmic and plasma membrane domains. The details of this ciliary gate are beginning to be resolved [17, 39]. Resendes et al. [73] reported that the basal body protein centrin-2 (Cetn2) interacts with the nucleoporin (Nup) 107–160 complex, an integral component of the nuclear pore. As with the yeast homolog Cdc31 [25, 74], Cetn2 is an integral component of the nuclear pore. The observation that nuclear transport proteins and nuclear localization-like sequences are involved in ciliary transport [19, 38, 83] and that blocking nuclear transport also blocks ciliary import [43, 82] suggests that nuclear pore/transport proteins were co-opted during ciliary evolution.

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That said, Breslow et al. [7] failed to find Nups localization or a functional role for Nups in the passive permeability barrier of primary (immotile) cilia. It is clear that this ciliary permeability barrier/transport system, which we refer to as the ciliary gate, is structurally and functionally distinct from the nuclear pore complex. In their studies of various motile cilia in the developing *Xenopus* embryo and cultured human retina pigment epithelial (RPE) cells, del Viso et al. [17] found evidence that the association of the inner ring (of the nuclear pore complex) Nups 188 and 93, but not other Nups examined (e.g., Nup63), are required for the formation of motile cilia in a range of cell types (the authors did not examine immotile, primary cilia). Their structural studies localized Nups 188/93 to the cilia base region, while 3D super-resolution microscopy in RPE cells revealed “the presence of two discrete structures, each composed of dozens of individual localization clusters that could not be resolved by confocal microscopy.” The overall distribution of cilia-associated Nup188/93 is distinctly different from the distribution of these proteins at the nuclear pore.

In the light of these studies, there is a general possibility that a subset of cilia-associated proteins contain localization/interaction sequences that can be recognized by cilia-associated structures, such as the Nups found in the pericentriolar material (PCM) region of the ciliary base, as well as components of the nuclear pore complex. The association of RAN complex components, important in active transport through nuclear pores [61], in the transition zone of cilia supports this model [19, 21]. RAN appears to be involved in “controlling injections of IFT proteins into the flagellar compartment” and “is, therefore, crucial to ciliogenesis” [53]. Similarly, transport of Kif17 [19, 29], retinitis pigmentosa 2 protein (RP2) [38], and Gli2 [89] into cilia is reported to be importin- β 2, but not importin- α / β 1, dependent (in the case of Gli2).

As examples, both the ciliary axoneme-associated radial spoke protein 3 (RSPH3–OMIM: 615876) [100] and the pericentriolar and ciliary basal body-associated protein pericentrin (PCNT: OMIM: 605925) [51] have been found to contain functional nuclear localization (NLS) and nuclear exclusion (NES) sequences. Similarly parafusin, a signaling scaffolding protein, has been found localized to the base of primary cilia in a variety of mammalian cell types and within the nuclei of fibroblasts [77]. The question arises then how common is it to find cilia-associated proteins in nuclei and what roles, if any, do they play there?

Characterizing cilia-associated proteins

Eukaryotic cilia are complex organelles composed of modified (double and triplet) microtubules and various associated proteins. Ishikawa et al. [41] identified 195

polypeptides in primary cilia, of which ~75% appeared to be present in motile cilia as well. Proximity labeling studies [57] have identified over 370 cilia-associated proteins. Boldt et al. [5] used an affinity proteomic-based study to identify 1319 cilia-associated proteins, 4905 inter-polypeptide interactions, and 52 molecular complexes. Such interaction complexity highlights the heterogeneous nature of ciliary functions in specific cell types and the likelihood that multiple evolutionary events along the vertebrate lineage have led to multiple functions for many of these proteins.

It is important to consider, however, that just because a protein is found associated with other ciliary components does not mean that its primary or sole function is ciliary. Many proteins are poorly studied, apparently for largely historical rather than functional criteria; Pandey et al. [68] coined the term “ignorome” for such proteins (and the genes that encode them). A dramatic example of the difficulty in defining gene product functions (one basis for the ignorome) is illustrated by the studies of Hutchinson et al. [40] who produced a minimal bacterial genome (JCV-syn3.0) containing 473 genes. Analysis of JCV-syn3.0 revealed an ignorome of 149 genes of unknown function (~32% of the total genome); these are genes required to produce a viable organism. At the same time, there is an increasing awareness that a number of proteins, originally identified as structural or functional components of one cellular system, can have quite distinct functions in another; they are what we might term “multi-taskers.” An example of such multitasking proteins is the catenins. Originally identified in the context of cadherin-based cell adhesion junctions [67], catenins were subsequently recognized to play important roles in the extracellular signaling-mediated regulation of gene expression [47, 54]. In this light, Kustatscher and Rappsilber [48] suggested that proteins have a certain degree of “fuzzy” distribution within cells. This notion, strengthened by proteomic analyses of subcellular compartments and organelle fractionation, indicates that stochastic intracellular movements are evolutionarily advantageous, as they increase the interaction potential of multitasker proteins. Contextually advantageous interactions are eventually “captured” by positive selection. This may well be the case for ciliary proteins in the nucleus, where their shared functions within related structures put them in position to acquire novel roles under specific circumstances.

One approach to defining the extent to which cilia-associated proteins are localized to nuclei involves mass spectrometric–proteomic studies of isolated nuclei. There are often technical obstacles to overcome in such an analysis, including the possibility of protein leakage and redistribution during nuclear isolation (see [55]). In their study Wühr et al. [99] exploited unique aspects

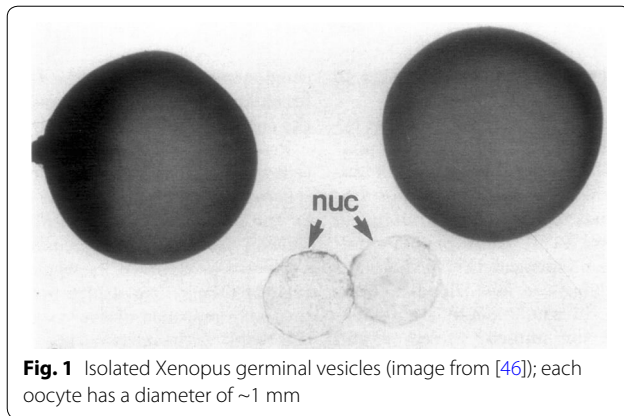


Fig. 1 Isolated *Xenopus* germinal vesicles (image from [46]); each oocyte has a diameter of ~1 mm

of the *Xenopus laevis* oocyte nucleus (the germinal vesicle), specifically its large size (~400 μm in diameter) and the ability to isolate intact germinal vesicles rapidly from late stage oocytes (Fig. 1) [46]. At the same time, oocytes are not known to express cilia of any kind, which limits the analysis to those proteins expressed during later stages of oogenesis. Wühr et al. characterized proteins based on their “relative nuclear concentration” (RNC), defined as the ratio of concentrations in the nucleus to the concentrations in nucleus plus the cytoplasm¹; the RNC ranges from 1 for a totally nuclear protein to 0 for a completely cytoplasmic (non-nuclear) protein. Of course polypeptides that reside in the nucleus are synthesized in the cytoplasm, nuclear isolation is not completely “clean” (even with isolated germinal vesicles), and a number of proteins are known to shuttle between nuclear and cytoplasmic compartments, so most RNC values are intermediate between 0 and 1. Nevertheless, it is possible to distinguish classes of proteins that appear to be excluded from nuclei and confined to the cytoplasm (value of RNC <0.1) from those that have a significant nuclear localization signal (RNC >0.35).

To estimate the number of cilia-associated proteins localized to nuclei, we used the Wühr et al. dataset together with the “gold standard” set of ~300 cilia-associated polypeptides described by van Dam et al. [92]¹; to this database, we added polypeptides known to be involved in ciliary function, i.e., *C2orf59* [42], *Cetn2* [81, 90, 101], and *EFHC1* and *EFHC2* (see [103]), for a total of 307 polypeptides. Of these 307 “gold standard” ciliary proteins, Wühr et al. report data on 118; of these, 30 (~25%) have RNC values of >0.35 (Table 1) and so are expected to have a significant nuclear presence. Other 29 polypeptides (~24%) have RNC values between 0.35 and 0.1 and may well be able to enter the nuclei. Within subgroups of related proteins, such as the interflagellar

transport polypeptides (IFTs), most appear exclusively cytoplasmic, but a few (e.g., IFT27) (Table 1) seem to be present at significant levels within the germinal vesicle. Similarly, the radial spoke protein *RSPH4A* (OMIM: 612647) has an RNC value of 0.9, higher than the known transcription factor *SOX13* (0.84), suggesting that *RSPH4A* is strongly concentrated in the germinal vesicle. In contrast, β -catenin (*CTNNB1*), which is well known to be able to enter the nuclei in response to canonical Wnt signaling (see [54]), appears to be exclusively cytoplasmic (RNC = 0.031) in the oocyte.

In a related set of unpublished studies, “nuclear” fractions were prepared from human SH-SY5Y neuroblastoma cells and analyzed by mass spectrometry (Additional file 1); of the 307 “gold standard” ciliary proteins, 93 were found within this nuclear (Table 2) fraction, including 15 that also had a “nuclear presence” in the *Xenopus* germinal vesicle dataset (Additional file 1). Together these data support the hypothesis that a significant percentage of the polypeptides linked to cilia formation, function, and phenotypic defects appear to be able to enter, and potentially play functional roles, in the nucleus. At this point, we describe a few of the best characterized of these nuclear functions.

Examples of cilia-associated proteins with known nuclear roles

Nephronophthisis (NPH) is an autosomal recessive cystic kidney disease characterized by inflammation and scarring (fibrosis) that compromises kidney function and leads, over time, to end-stage renal disease [98]. Many of the genes linked to NPH, encoding polypeptides known as nephrocystins or NPHPs, have been found to encode proteins localized to or involved in primary cilia formation or cilia-dependent signaling (both Wnt and Shh) which presumably explains phenotypic effects on other organ systems. Where other organs are involved, the disease is termed NPH-related ciliopathy [98]. Of the NPHP-associated genes, a number have been found to have direct or indirect nuclear roles. For example, **NPHP4** (nephrocystin-2: OMIM 607215) interacts with and inhibits the LATS1 kinase, leading to the nuclear accumulation of the transcriptional co-activators YAP and TAZ, two proteins involved in the HIPPO (hypoxia) signaling system [33]. **NPHP9** (also known as *NEK8*—OMIM 609799) has been found to interact with TAZ, leading to the nuclear localization of the NPHP9–TAZ complex [34]. TAZ is normally exported to the cytoplasm through interactions with 14-3-3 proteins; NPHP9 appears to compete with 14-3-3 proteins for TAZ binding. NPHP4 inhibits LATS1 phosphorylation of TAZ, which reduces its affinity for 14-3-3 proteins. NPHP4 also interacts with the ubiquitin ligase JADE1

¹ Website accessible here: <http://www.syscilia.org/goldstandard.shtml>.

Table 1 Ciliary proteins with a nuclear presence

	Nuclear + cytoplasmic >0.2	Ambiguous between 0.2 and 0.1	Cytoplasmic >0.1
ARL3	ADP-ribosylation factor-like protein 3	Interactor with RP2 and PDE δ . Involved in trafficking of vesicles from the Golgi to the cilium, especially; farnesylated cargo in association with PDE δ and RPGR; and myristoylated cargo in association with UNC119 and RP2	RNC = 0.51 (nuc + cyto)
ARL6	ADP-ribosylation factor-like protein 6	Mutated in Bardet-Biedl syndrome (type 3) and retinitis pigmentosa (type 55). At the ciliary gate, it regulates Wnt signaling. Functions with BBSome to coat proteins for trafficking to the cilium basal body, cilium, cytosol, transition zone	RNC = 0.49 (nuc + cyto)
ATXN10	Ataxin 10	Mutated in 1 NPHP family (splice-site mutation). Forms complex with NPHP5 and 6 at the basal body	RNC = 0.48 (nuc + cyto)
C21orf59^a	Jaffe et al. [42] c21orf59/kurly controls both cilia motility and polarization		RNS = 0.48 (nuc + cyto)
C8orf37	Basal body, ciliary root	Mutated in cone-rod dystrophy (type 16) and retinitis pigmentosa (type 64). Localizes to basal body in cultured RPE cells and Basal body and ciliary rootlet in mouse photoreceptors	RNS = 0.36 (nuc + cyto)
CETN2^a	Centrin-2		RNS = 0.55/0.58 (nuc + cyto)
CETN3^a	Centrin-3		RNS = 0.33 (nuc + cyto)
CTNNB1	Catenin beta-1	Involved in regulation of PKD1 and PKD2 expression. Nek2 substrate involved in centrosome separation, along with rootletin (CROCC). Facilitator of canonical Wnt signalling pathway. Many links between cilia and Wnt signaling centrosome	RNS = 0.031 (cytoplasmic^a)
DNAL1	Dynein light chain 1, axonemal	Mutated in primary ciliary dyskinesia (type 16) Component of outer dynein arms. Axoneme	RNS = 0.49 (nuc + cyto)
DPCD	Deleted in primary ciliary dyskinesia	Deleted in a mouse model of primary ciliary dyskinesia	RNS = 0.29 (nuc + cyto)
EFHC2^a	EF-hand domain-containing family member C2		RNS = 0.47 (nuc + cyto)
HEATR2	HEAT repeat-containing protein 2	Mutation linked to premature centromere division (PCD), presumably involved in dynein arm transport or assembly	RNS = 0.63 (nuc + cyto)
HSPA8	Heat shock cognate 71 kDa protein	Chaperone of the IFT together with DnajB6	RNS = 0.49 (nuc + cyto)
HSPB11	Heat shock protein beta-11	IFT25, part of IFT-B complex. Forms a complex with IFT27. External submission. Cilium, IFT	RNS = 0.48 (nuc + cyto)
IFT27	Intraflagellar transport protein 27	Component of IFT complex B. Rab-like small G protein basal body, cilium, IFT	RNS = 0.45 (nuc + cyto)
KIF17	Kinesin-like protein KIF17	Kinesin 2 motor, active in anterograde IFT ciliary tip	RNS = 0.21 (nuc + cyto)
LRRC6	Protein TILB homolog	Essential for proper axonemal assembly of inner and outer dynein arms, causes PCD. Cilium	RNS = 0.34 (nuc + cyto)
MAPRE1 (EB1)	MT-associated protein RP/EB family 1	MT plus-end-tracking protein. promotes ciliogenesis. Centrosome, golgi	RNS = 0.38 (nuc + cyto)
MNS1	Meiosis-specific nuclear structural protein 1	Mns1 $-/-$ mice have short, immotile sperm flagella, situs defects, and hydrocephalus. Protein localized along flagellum. Knockdown in IMCD3 and 3T3 cells causes Hh signaling defects axoneme	RNS = 0.37 (nuc + cyto)
NME7	Nucleoside diphosphate kinase 7	IFT transport and signaling defects after knockdown	RNS = 0.21 (nuc + cyto)
NME8	Thioredoxin domain-containing protein 3	The sea urchin ortholog of this gene encodes a component of sperm outer dynein arms, and the protein is implicated in ciliary function. Mutations in this gene are implicated in primary ciliary dyskinesia type 6	RNS = 0.39 (nuc + cyto)

Table 1 continued

NPHP1	Nephrocystin-1 Known ciliopathy gene JBTS4, NPHP1, SLS1 transition zone	RNS = 0.36 (nuc + cyto)
NUP214	Nuclear pore complex protein Nup214 Part of ciliary pore complex transition zone	RNS = 0.45 (nuc + cyto)
NUP35	Nucleoporin NUP35 Nucleoporin 35 kDa, part of ciliary pore complex transition zone	RNS = 0.47 (nuc + cyto)
NUP37	Nucleoporin NUP37 Part of ciliary pore complex transition zone	RNS = 0.56 (nuc + cyto)
NUP62	Nuclear pore glycoprotein p62 Part of ciliary pore complex transition zone	RNS = 0.38 (nuc + cyto)
NUP93	Nuclear pore complex protein Nup93 Part of ciliary pore complex transition zone	RNS = 0.45 (nuc + cyto)
OCRL	Oculocerebrorenal syndrome of Lowe Linked to Lowe Syndrome. Involved in assembly of primary cilia, involved in Rab8n-dependent protein trafficking to the cilium	RNS = 0.24 (nuc + cyto)
ORC1	Origin recognition complex subunit 1 Linked to Meier-Gorlin syndrome (MGS), a disorder conferring microcephaly, primordial dwarfism, underdeveloped ears, and skeletal abnormalities. knockdown affects ciliogenesis and Hh signaling	RNS = 0.23 (nuc + cyto)
PAFAH1B1	Platelet-activating factor acetylhydrolase IB alpha LIS1 Mammalian Lis1 localizes to motile cilia in trachea and oviduct, but is absent from non-motile primary cilia axoneme	RNS = 0.28 (nuc + cyto)
PDE6D	cGMP 3',5'-cyclic phosphodiesterase subunit delta Part of the ARL13B, INPP5E, and CEP164 network	RNS = 0.40 (nuc + cyto)p
PLK1	Serine/threonine-protein kinase PLK1 Localizes to TZ and induces phosphorylation of NPHP1 transition zone	RNS = 0.39 (nuc + cyto)
RABL5	Rab-like protein 5 IFT22, component of IFT complex B cilium, IFT	RNS = 0.33 (nuc + cyto)
RILPL2	RILP-like protein 2 Rab effector. Regulates cilium membrane content. Cilium, basal body	RNS = 0.50 (nuc + cyto)
RSPH4A	Radial spoke head protein 4 homolog A Defective Hh signaling but no structural cilia defects after knockdown. Mutations in RSPH4A cause primary ciliary dyskinesia, with typical respiratory features, but without situs abnormalities. These findings suggest that radial spoke proteins are not essential for embryonic nodal ciliary function, which is important in the determination of left-right axis development cilium, axoneme	RNS = 0.90 (most nuclear)
RSPH9	Radial spoke head protein 9 homolog Mutations in RSPH9 linked to primary ciliary dyskinesia, with typical respiratory features, but without situs abnormalities. These findings suggest that radial spoke proteins are not essential for embryonic nodal ciliary function, which is important in the determination of left-right axis development structural cilia defects of primary cilia after knockdown in 3 different murine cell lines cilium, axoneme	RNS = 0.44 (nuc + cyto)
SNX10	Sorting nexin-10 (Fragment) Regulates ciliogenesis Centrosome	RNS = 0.51 (nuc + cyto)
SSNA1	Sjogren syndrome nuclear autoantigen 1 siRNA knockdown in 3 mouse cell lines perturbs receptor transport into cilium and HH signaling	RNA + 0.50 (nuc + cyto)
STK38L	Serine/threonine-protein kinase 38-like Phosphorylates Rabin8. Mutated in canine retinal degeneration. Cytosol	RNA = 0.34 (nuc + cyto)
SUFU	Suppressor of fused homolog Localizes to ciliary tip together with GLI transcription factors. Role in Hh signaling ciliary tip	RNS = 0.24 (nuc + cyto)
TEKT4	Tektin-4 Axonemal protein required for flagella motility in mouse sperm. Expression reduced in inasthenozoospermic men. Cilium	RNS = 0.35 (nuc + cyto)

Table 1 continued

TNPO1	Transportin-1	RNS = 0.44 (nuc + cyto)
	Importin beta 2. Regulates entry of RP2 and kinesin motor into cilium axoneme	
TRAPPC3	Trafficking protein particle complex subunit 3	RNS = 0.30 (nuc + cyto).
	Required for Rabin8 centrosome trafficking and ciliogenesis basal body, centrosome	
TTK	Dual specificity protein kinase TTK	RNS = 0.33 (nuc + cyto)
	Negatively regulates ciliogenesis. Centrosome	
WDR19	WD repeat-containing protein 19	RNS = 0.22 (nuc + cyto)
	Mutations in WDR19 associated with ciliopathies nephronophthisis (NPHP13), Jeune and Sensenbrenner syndromes (ATD5), IFT complex A component (aka IFT144) cilium, IFT	

Xenopus Proteomic data from Wuhr et al. ciliary proteins (and curator notes) from: <http://www.syscilia.org/goldstandard.shtml>

^a Indicates known ciliary protein added to list of ciliary proteins

Table 2 Cilia-associated proteins found in SHSY5Y-NUCLEAR dataset

ARF4	ARL3 ^a	ARL6 ^a	ASAP1	ATXN10 ^a	Cetn2 ^a
Cetn3	CEP135	CEP250	CEP41	CEP89	CEP97
CP110	CROCC	CTNNB1 ^b	DNAH1 ^a	DNAH11	DNAH5
DPCD	DPYSL2	DYNLT1	EXOC3	EXOC4	EXOC5
EXOC6	FLNA	GSK3B	HEATR2 ^a	HSPA8 ^a	HTT
IFT27 ^a	IFT52	IFT81	INVS	KIF3A	KIF3C
MLF1	NGFR	NME7	MAPRE1 ^a (EB1)	LZTFL1	NPHP4
NUP214 ^a	NUP35 ^a	NUP37	NUP62 ^a	NUP93 ^a	OCRL
ODF2	ORC1	PAFAH1B1	PARD3	PCM1	PDE6 D ^a
PHF17	PKD2	PLK1 ^a	RAB11A	RAB23	RAB8A
RAN	RANBP1	REF3	RP2	SEPT2	SEPT7
SGK196	SNAP25	STK38L	SYNE2	TNPO1 ^a	TRAPPC10
TRAPPC3	TRIM32	TRIP11	TTC21B	TTC30A	TTC8
TUBA1C	TUBA4A	TUBB2A	TUBB2B	TUBB3	TUBE1
TUBGCP2	TUBGCP3	TUBGCP4	VDAC3	XPNPEP3	

^a Indicates also found to have a nuclear presence in the Xenopus dataset (Table 1)

^b JUP/Plakoglobin also present

(another ciliary and nuclear component), an interaction that appears to stabilize JADE1 and lead to its nuclear localization where it acts to destabilize β -catenin and inhibit canonical Wnt signaling [6]. NPHP4 itself appears to be primarily perinuclear. The absence of NPHP4 activity leads to an increase in Wnt signaling and subsequent cyst formation in the kidney [6]. Studies in the zebrafish support this mechanism [9]. **NPHP7** (OMIM: 611498) is a GLI-like zinc finger transcription factor involved in the regulation of mesenchymal–epithelial cell behavior, inhibiting the expression of genes such as Snail1 and Wnt4 [72]. Mutations in NPHP7 have been associated with NPH-related ciliopathy [35]. Morpholino-mediated downregulation of NPHP7 has been found to influence the formation of immotile motile cilia and a number of associated phenotypes, including cystic pronephros and

ciliary motility in zebrafish [45, 71], presumably due to effects on gene expression. **NPHP10** (OMIM: 613524) localizes to nuclear foci with a number of DNA damage response proteins, including the centrosomal protein CEP164 (OMIM: 614848) [11].

Oral-facial-digital 1 (OFD1) syndrome

OFD1 (OMIM: 311200) is one of an array of nine phenotypically similar disorders. OFD1 is an X-linked dominant disease associated with facial malformations as well as cystic kidneys [22]. OFD1 has been linked to the gene OFD1 (previously known as Cxorff5) (OMIM: 300170). In a mouse model OFD1 has been associated with defects in primary cilia formation and left–right axis formation [23]. Subsequent studies indicate that OFD1 has a nuclear presence, and plays a role in chromatin remodeling [32] and in double-stranded DNA break repair, mediated by the TIP60 complex [1].

Bardet-Biedl Syndrome

Bardet–Biedl syndromes are group of pleiotropic oligogenic ciliopathies [64] that have been linked to (at least) nineteen different genes with potentially etiological roles. Of these, **BBS1, 2, 4, 5, 6, 7, 8, and 10** have been found to enter the nucleus where they can influence gene expression through interactions with the polycomb group member protein RNF2 (Ring Finger Protein 4: OMIM: 602850) [30]. Both BBS1 (OMIM: 209901) and BBS11 (OMIM: 615988) have been reported to interact with and to alter the activity of NPHP7 [45, 71].

Centrins and pericentrin

Centrins (Cetn) are calmodulin-like proteins associated with centrosomes (microtubule-organizing centers) and the basal body regions of cilia. Two centrin subclasses have been identified, Cetn2-like and Cetn3-like [14, 28]. In the yeast *Saccharomyces cerevisiae*, there is a single centrin gene (Cdc31); the Cdc31 protein is found localized to nuclear pores in addition to the spindle pole body

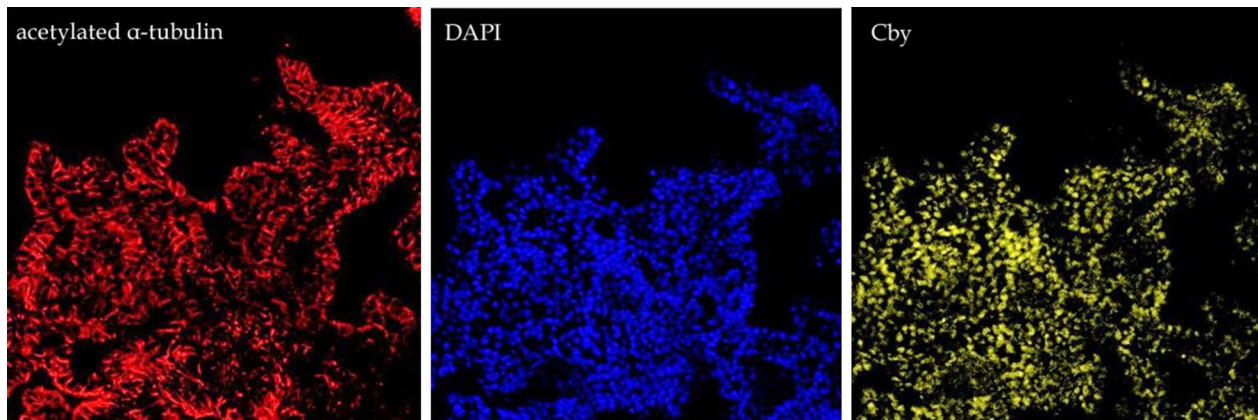


Fig. 3 Human iPSC-derived cerebral organoids were stained in whole-mount for acetylated α -tubulin (*lefthand panel*), the DNA marker DAPI (*central panel*), and Cby (*right hand panel*) using a rabbit anti-Cby antibody, supplied by Feng-Qian Li (University of Stony Brook) and described in Li et al. [50]

Inversin also interact with the paralogous protein plakoglobin (γ -catenin or JUP: OMIM: 173325) has not been reported.

Summary

The challenges in separating the ciliary and cytoplasmic from possible nuclear functions of cilia-associated proteins are similar to those faced in the analysis of the adhesive (cytoplasmic) and gene expression (nuclear) roles of β -catenin and γ -catenin (plakoglobin), which share many interaction partners [97]. The use of cytoplasmically anchored forms allowed us to conclude that γ -catenin's effects on β -catenin-mediated gene expression were indirect [56]. Given the shared mechanisms acting at nuclear pores and ciliary gates, a similar (or perhaps a more modern) strategy seems necessary to distinguish the ciliary and cytoplasmic from the non-ciliary, i.e., nuclear, functional roles of cilia-associated proteins.

Additional file

Additional file 1. Additional material.

Abbreviations

OMIM: Online Mendelian Inheritance in Man: <https://www.omim.org>; RNC: relative nuclear concentration; RPE cells: human retina pigment epithelial cells; NLS: nuclear localization sequence; NES: nuclear exclusion sequence.

Authors' contributions

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Competing interests

Both authors declare that they have no competing interests.

Availability of data

Results from the proteomic analysis of SH-SY5Y cells will be made publicly available through the Old Lab web server.

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