CHAPTER FIVE

Modeling Ciliopathies: Primary Cilia in Development and Disease

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Contents

Abstract

Primary (nonmotile) cilia are currently enjoying a renaissance in light of novel ascribed functions ranging from mechanosensory to signal transduction. Their importance for key developmental pathways such as Sonic Hedgehog (Shh) and Wnt is beginning to emerge. The function of nodal cilia, for example, is vital for

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breaking early embryonic symmetry, Shh signaling is important for tissue morphogenesis and successful Wnt signaling for organ growth and differentiation. When ciliary function is perturbed, photoreceptors may die, kidney tubules develop cysts, limb digits multiply and brains form improperly. The etiology of several uncommon disorders has recently been associated with cilia dysfunction. The causative genes are often similar and their cognate proteins certainly share cellular locations and/or pathways. Animal models of ciliary gene ablation such as *Ift88*, *Kif3a*, and *Bbs* have been invaluable for understanding the broad function of the cilium. Herein, we describe the wealth of information derived from the study of the ciliopathies and their animal models.

"This world, after all our science and sciences, is still a miracle; wonderful, inscrutable, magical and more, to whosoever will think of it." (Thomas Carlyle)

1. The Human Ciliopathies

The term "ciliopathy" has been coined to describe a class of rare human genetic diseases whose etiologies lie in defective cilia. In vertebrates, cilia are present in nearly all organs and cell types; however, amongst invertebrates they are confined to the sensory neurons that sense chemical stimuli, changes in environment and even vibration (Evans *et al.*, 2006). Cilia fall into two broad categories: those that are motile and those that are not. As the name would suggest, motile cilia and flagella (which share structural identity) are important for cell motility or for establishing fluid flow across their surface. For example, cells lining the respiratory tract, oviducts, epididymis, and ependymal surface of the brain display large clusters of motile cilia, which beat in concert to generate a wave-like motion. In contrast, the ubiquitous immotile or "primary" cilium, long regarded as a vestigial remnant of its motile cousin, is sessile in nature and present as a solitary extension of the plasma membrane. It is now accepted that primary cilia serve a broad sensory purpose in transducing extracellular information to the cell interior. Central to this is their role in several signal transduction pathways including the noncanonical Wnt/planar cell polarity (PCP) pathway and the Hedgehog (HH) pathway as well as for regulation of intracellular calcium concentration.

In the past few years, appreciation of ciliary function has led to reanalysis of a number of human syndromes. Because of a growing wealth of cellular and molecular evidence for the role of cilia in development and normal human physiology, a number of diseases that had previously been loosely associated due to shared clinical features (such as Bardet-Biedl and Alström syndromes) can now confidently be grouped together under the classification of a ciliopathy. This progress has also revealed some surprising new

interrelationships amongst diseases (such as Bardet-Biedl and Meckel syndromes). What now links these individual disorders is the findings that the gene products, known to cause the disease if mutated, are localized to the cilia or their anchoring structure, the basal body, and play some role in its function. Consistent with their broad tissue and cellular distribution, it is now recognized that defects in cilia give rise to an eqaully broad but consistent range of phenotypes in mammals that are associated with organ-specific disorders such as polycystic kidney disease (PKD) as well as broad, pleiotropic syndromes. These syndromes and their main clinical features are summarized in Table 5.1. This table encapsulates most considerable clinical overlap between these syndromes; for instance most display renal cystic and hepatobiliary disease, some have laterality defects and retinal degeneration and several have polydactyly. It is also possible to further subdivide these disorders into those with skeletal involvement (JATD, OFD1, EVC) and those without (BBS, NPHP, MKS, JBTS, ALMS) as illustrated in Fig. 5.1. Although the individual disorders that make up the ciliopathies are thought of as rare genetic diseases, when viewed collectively, their prevalence rate could be as high as ~1 in 2000 [based on three common disease traits: renal cysts (1 in 500 adults), retinal degeneration (1 in 3000), and polydactyly (1 in 500)].

Although we depend on experimental models for our understanding of the biological processes underpinning disease, perhaps the most informative and diverse of all disease models are *Homo sapiens* themselves. Thus, this review will discuss the role of primary cilia in relation to human health and disease (with only limited reference to motile cilia defects) and with reference to relevant murine and other animal models that have informed and helped us to understand their etiology. The ciliopathies and the loci/genes that underlie their manifestation are summarized in Table 5.2.

2. Bardet-Biedl Syndrome

Bardet-Biedl syndrome (BBS) is a heterogeneous pleiotropic disorder inherited in a mainly recessive manner. Despite the growing list of syndromes now classified as a ciliopathy, it has been BBS that has led the quest to understand the pathomechanisms of this class of disease. Its clinical features include retinal degeneration, cognitive impairment, obesity, renal cystic disease, polydactyly, and occasionally *situs inversus*. It was these observations, in light of emerging evidence of the etiology of left–right (LR) asymmetry (discussed further below) that implicated cilia in the pathogenesis of BBS (Ansley *et al.*, 2003). Currently, twelve genes (*BBS1–12*) have been identified (summarized in Table 5.2) although, to date, no consistent phenotype–genotype correlations have been established (Ansley *et al.*, 2003; Badano *et al.*, 2003; Chiang *et al.*, 2004, 2006; Fan *et al.*, 2004;

Table 5.1 Common clinical features of the ciliopathies

Feature	BBS	MKS	JBTS	NPHP	SLSN	JATD	OFD1	EVC	ALMS	PKD
Renal cysts	✓	✓	✓	✓	✓	✓	✓		✓	✓
Hepatobiliary disease	✓	✓	✓	✓	✓	✓	✓		✓	✓
Laterality defects	✓	✓		✓		✓				
Polydactyly	✓	✓	✓			✓	✓	✓		
Agenesis of corpus callosum	✓	✓	✓			✓	✓		✓	
Cognitive impairment	✓	✓	✓			✓	✓	✓		
Retinal degeneration	✓	✓	✓		✓	✓			✓	
Posterior fossa defects/ encephalocoele	✓	✓	✓			✓		✓		
Skeletal bone defects						✓	✓	✓		
Obesity	✓								✓	

BBS: Bardet-Biedl syndrome; MKS: Meckel syndrome; JBTS: Joubert syndrome; NPHP: Nephrophthisis; SLSN: Senior-Løken syndrome; JATD: Jeune syndrome; OFD1: Oro-facial-digital syndrome type 1; EVC: Ellis van Creveld syndrome; ALMS: Alström Syndrome; PKD: Polycystic kidney disease

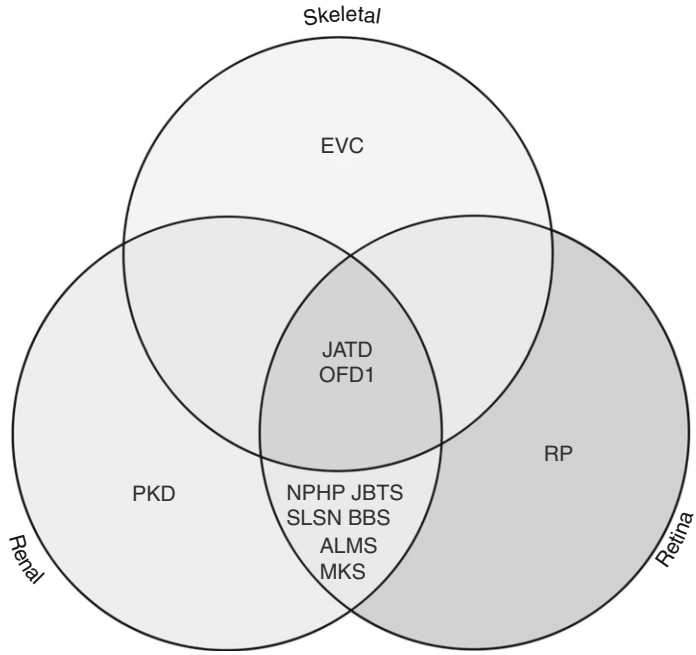

Figure 5.1 Clinical overlap between the ciliopathies. As outlined in Table 5.1, the ciliopathies can display a broad range of clinical features. Sorting the individual syndromes according to the occurrence or not of three features (renal cysts = green; retinal degeneration = blue; skeletal bone defects = yellow) helps to illustrate the commonalities and differences between these disorders. *Abbreviations*: BBS, Bardet-Biedl syndrome; MKS, Meckel syndrome; JBTS, Joubert syndrome; NPHP, nephrophthisis; SLSN, Senior-Løken syndrome; JATD, Jeune syndrome; OFD1, Orofacial–digital syndrome type 1; EVC, Ellis-van Creveld syndrome; ALMS, Alström syndrome; RP, retinitis pigmentosa; PKD, polycystic kidney disease.

Katsanis *et al.*, 2000; Li *et al.*, 2004; Mykytyn *et al.*, 2001, 2002; Nishimura *et al.*, 2001, 2005; Slavotinek *et al.*, 2000; Stoetzel *et al.*, 2006). The link with cilia was not at first apparent owing to a lack of sequence-based information for the early BBS genes. For example, the amino acid sequence of *BBS6/MKKS*, *BBS10*, and *BBS12* have strong homology with the type 2 group of chaperones, which initially suggested a role in protein folding but gave little insight into their actual, though still not clearly determined, function (Kim *et al.*, 2005; Stoetzel *et al.*, 2006, 2007). Of the remaining genes, only *BBS3/ARL6* (a member of the Ras superfamily of small GTP-binding proteins) and *BBS11/TRIM32* (an E3 ubiquitin ligase) encode proteins of known function. As indicated in Table 5.2, knockout and conditionally mutant mice have now been generated for a number of BBS genes. An interesting aspect of these mutants is that they do not fully phenocopy the cardinal features seen in BBS patients. For instance,

Table 5.2 Summary of ciliopathy genes associated with human syndromes

Human Syndrome	HUMAN Gene	*also known as*	Associated Ciliopathy	Locus and OMIM	Protein	Murine model	Key References
Alström Syndrome OMIM 203800	***ALMS1***		**Type 2 diabetes**	*2p13* **606844*	**Alström syndrome protein 1**	*Foz Alms null*	Cano *et al.* (2004, 2006), Caridi *et al.* (1998), Caspary *et al.* (2007), Chang *et al.* (2006), Porter *et al.* (1999)
Bardet-Biedl Syndrome OMIM 209900	***BBS1***			*11q13* **209901*	**Bardet-Biedl syndrome 1 protein**	*Bbs1 (M390R)* *Bbs1 null*	Atala *et al.* (1993), Bergmann *et al.* (2008), Mollet *et al.* (2005), Qian *et al.* (1997)
	BBS2			*16q21* **606151*	**Bardet-Biedl syndrome 2 protein**	*Bbs2 null*	Arts *et al.* (2007), Ibraghimov-Beskrovnaya *et al.* (2008), Qin *et al.* (2001)
	ARL6	*BBS3*		*3p12-q13* **608845*	**ADP-ribosylation factor-like protein 6** Bardet-Biedl syndrome 3 protein		Barr *et al.* (1999), Beales *et al.* (2007)
	BBS4			*15q22.3* **600374*	**Bardet-Biedl syndrome 4 protein**	Bbs4 null	Attanasio *et al.* (2007), Bergmann *et al.* (2008), Huangfu *et al.* (2003), Hughes *et al.* (1995), Ingham *et al.* (2008), Qin *et al.* (2001), Mollet *et al.* (2005), Rahmouni *et al.* (2008)

	BBS5			*2q31* **603650*	**Bardet-Biedl syndrome 5 protein**		Baala *et al.* (2007)
	MKKS	*BBS6*	**McKusick-Kaufman Syndrome** OMIM 236700	*20p12* **604896*	**Bardet-Biedl syndrome 6 protein** McKusick-Kaufman/Bardet-Biedl syndromes putative chaperonin	Bbs6 null Mkks null	Badano *et al.* (2003, 2006), Benzing *et al.* (2001), Huangfu *et al.* (2003), Mollet *et al.* (2005)
	BBS7			*4q27* **607590*	**Bardet-Biedl syndrome 7 protein**		Beales *et al.* (1999)
	TTC8	*BBS8*		*14q32.11* **608132*	**Bardet-Biedl syndrome 8 protein** Tetratricopeptide repeat protein 8		Alberts *et al.* (2008)
	PTHB1	*BBS9*		*7p14* **607968*	**Bardet-Biedl syndrome 9 protein** Parathyroid hormone-responsive B1 gene protein		Arsov *et al.* (2006)
	BBS10			*12q* **610148*	**Bardet-Biedl syndrome 10 protein**		Ansley *et al.* (2003), Rana *et al.* (2004)
	TRIM32	*BBS11*		*9q33.1* **602290*	**Tripartite motif-containing** Bardet-Biedl syndrome 11 protein		Andersen *et al.* (2003)
	BBS12			*4q27* **610683*	**Bardet-Biedl syndrome 12 protein**		Berbari *et al.* (2008)
Modifies BBS phenotypes	***CCDC28B***			**610162*			Peters *et al.* (1999)

(*continued*)

Table 5.2 (*continued*)

Human Syndrome	HUMAN Gene	*also known as*	Associated Ciliopathy	Locus and OMIM	Protein	Murine model	Key References
					Coiled-coil domain-containing protein 28B		
Ellis van Creveld syndrome OMIM 225500	***EVC***			*14p16* **604831*	**Ellis-van Creveld syndrome protein**	*Evc null*	Robert *et al.* (2007), Roepman *et al.* (2005)
	LBN	*EVC2*		*14p16* **607261*	**Limbin**		Rohatgi *et al.* (2007), Ross *et al.* (2005)
Jeune syndrome OMIM 208500	***unknown***	*JATD1*		*15q13*	**n/a**		Ruiz-Perez *et al.* (2007)
Jeune syndrome OMIM 611263	***IFT80***	*JATD2*		*3q24-26* **611177*	**Intraflagellar transport protein 80 homolog**		Ferrante *et al.* (2006), Guay-Woodford *et al.* (1995)
Joubert syndrome OMIM 213300 **including Type B/ CORS and JSRD**	***unknown***	*JBTS1*		*9q34.3*			Ruiz-Perez *et al.* (2000)
	unknown	*JBTS2*		*11p12-q13.3* *608091			Galdzicka *et al.* (2002), Saar *et al.* (1999)
	AHI1	*JBTS3*		*6q23.3* **608894*	**Abelson helper integration site 1 protein homolog** Jouberin		Garcia-Garcia *et al.* (2005), Gattone *et al.* (1996), Gerdes *et al.* (2007), Germino and Somlo (1993), Giorgio *et al.* (2007), Giusto and Sciubba (2004), Saburi *et al.* (2008), Sage (1991)

	RPGRIP1L	*FTM* *JBTS7* *NPHP8* *MKS5*	**MKS Type 5** OMIM 611561 **NPHP Type 8** OMIM n/a	*16q12.2* *****610937	**RPGR-interacting protein 1-like protein** Protein fantom	*Ft*	Eggenschwiler and Anderson (2007), Eggenschwiler *et al.* (2006), Eichers *et al.* (2006), Eley *et al.* (2008), Saalonen and Paavola (1998)
	ARL13B	*JBTS8*		3p12.3-q12.3 608922*****	**ADP-ribosylation factor-like protein 13B**	*hnn* *scorpion (zebrafish)*	Grace *et al.* (2003), Green *et al.* (1989), Gretz *et al.* (1996), Guay-Woodford *et al.* (1996)
Meckel syndrome OMIM 249000	***MKS1***	*BBS13*	**BBS Type 13** OMIM 609883	*17q23* *****609883	**Meckel syndrome type 1 protein**		Salonen (1984)
	TMEM67	*MKS3* *JBTS6*	Originally used as model for human **ARPKD** OMIM 263200 **JBTS Type 6** OMIM 609884	*Chr. 8q* *****609884	**Meckelin** Meckel syndrome Type 3 protein Transmembrane protein 67	*wpk* (RAT)	Ferland *et al.* (2004), Ferrante *et al.* (2001), Gonzalez-Perrett *et al.* (2001), Sanzen *et al.* (2001)
	CC2D2A	*MKS6*		*4p15.3* *****612013	**Coiled-coil and C2 domain-containing protein 2A**		Ferrante *et al.* (2006), Flaherty *et al.* (1995), Fliegauf *et al.* (2003)
Nephrono-phthisis (Juvenile) OMIM 256100 **(Infantile)**	***NPHP1***	*JBTS4*	**JBTS Type 4** OMIM 609583 **SLSN Type 1** OMIM 266900 **NPHP Type 1** OMIM 607100	2q13 *****607100	**Nephrocystin-1** Juvenile nephronophthisis 1 protein	*Nphp1 null*	Sayer *et al.* (2006), Schafer *et al.* (1994), Schneider *et al.* (1996), Schrick *et al.* (1995)

(*continued*)

Table 5.2 (*continued*)

Human Syndrome	HUMAN Gene	*also known as*	Associated Ciliopathy	Locus and OMIM	Protein	Murine model	Key References
OMIM 602088	***INVS***	*NPHP2*		9q31 *243305	**Inversin** Nephrocystin-2	*inv*	Davenport *et al.* (2007), Kim *et al.* (2004), Marszalek *et al.* (1999), Schuermann *et al.* (2002), Shah *et al.* (2008)
	NPHP3		Originally used as model for human **ADPKD** OMIM 173900 **SLSN Type 3** OMIM 606995	3q22 *608002	**Nephrocystin-3**	*pcy* *Nphp3 null*	Dawe *et al.* (2007), Deane *et al.* (2001), Sheng *et al.* (2008), Shillingford *et al.* (2006)
	NPHP4		**SLSN Type 4** OMIM 606996	1p36 *607215	**Nephrocystin-4** Nephroretinin		Doudney and Stanier (2005), Signor *et al.* (1999), Simons *et al.* (2005), Slavotinek *et al.* (2000), Smith *et al.* (2006)
	IQCB1	*NPHP5*	**SLSN Type 5** OMIM 609254	3q21.1 *609237	**IQ calmodulin-binding motif-containing protein 1** Nephrocystin-5	*n/a*	Drummond *et al.* (1998)
	CEP290	*MKS4* *JBTS5* *LCA10* *BBS14* *NPHP6* *SLSN6*	**SLSN Type 6** OMIM 610189 **MKS Type 4** OMIM 611134 **LCA Type 10** OMIM 611755 **BBS Type 14** OMIM 209900 **JBTS Type 5** OMIM 610188	12q21.3 *610142	**Centrosomal protein of 290 kDa** Nephrocystin-6	*rd16*	Drummond (2005), Eberhart *et al.* (2006), Edelstein (2008), Sohara *et al.* (2008)

	GLIS2	*NPHP7* *NKL*		16p13.3 *608539	**Zinc finger protein GLIS2 (GLI-similar 2)** Neuronal Krueppel-like protein	*Glis2 null*	Edelstein (2008), Somlo and Ehrlich (2001), Spassky *et al.* (2008)
	NEK8	*JCK* *NPHP9*	originally used for model of human **ARPKD** OMIM 263200 **NPHP Type 9** OMIM 256100	17q11.1 *609799	**Serine/threonine-protein kinase Nek8** Never in mitosis A-related kinase 8	*jck*	Eley *et al.* (2005), Nonaka *et al.* (1998), Patel *et al.* (2008), Stamataki *et al.* (2005), Starich *et al.* (1995)
Obesity	***TUB***		maturity-onset obesity and insulin resistence	11p15.5 *601197	**Tubby protein homolog**	*tub* *Rd5 gene in mouse*	Stoetzel *et al.* (2006, 2007)
	FTO		Type II diabetes obesity	16q12.2 *610966	**Protein fatso** Fat mass and obesity-associated protein	*Ft*	Sulik *et al.* (1994), Sullivan-Brown *et al.* (2008)
Oro-facial-digital syndrome OMIM 311200	***OFD1***			Xp22.3-p22.2 *300170	**Oral-facial-digital syndrome 1 protein**	Ofd1 null and conditional alleles	Guay-Woodford (2003), Habas *et al.* (2003), Han *et al.* (2008)
Polycystic Kidney Disease **(autosomal dominant)** OMIM 173900	***PKD1***			16p13.3-p13.12 *601313	**Polycystin-1** Autosomal dominant polycystic kidney disease protein 1	*Pkd null and various conditional alleles*	Chiang *et al.* (2004), Nauta *et al.* (1993), Sun *et al.* (2004)
	PKD2			4q21-q23 *173910	**Polycystin-2** Autosomal dominant polycystic kidney disease type II protein Polycystwin	*Pkd null and various conditional alleles* *Pkd2*$^{WS25/-}$	Chiang *et al.* (2004), Chizhikov *et al.* (2007), Nauli *et al.* (2003), Nauta *et al.* (1993), Ocbina and Anderson (2008), Supp *et al.* (1997, 1999), Takahashi *et al.* (1991)

(*continued*)

Table 5.2 (*continued*)

Human Syndrome	HUMAN Gene	*also known as*	Associated Ciliopathy	Locus and OMIM	Protein	Murine model	Key References
***Polycystic Kidney Disease* (autosomal recessive)** OMIM 263200	***PKHD1***		initially proposed as model for **ADPKD** OMIM 173900 because of late onset of disease in rat.	6p21.1-p12 *606702	**Fibrocystin** Polycystic kidney and hepatic disease 1 protein Polyductin	*pck (rat)*	Cole *et al.* (1998), Nauta *et al.* (1997), Takahashi *et al.* (1986), Takeda *et al.* (1999, 2002)
Primary Ciliary Dyskinesia OMIM 242650	***DNAHC11***	*LRD*		7p21 *603339	**Axonemal dynein heavy chain isotype 11** left/right-dynein	*iv*	Kaspareit-Rittinghausen *et al.* (1990), Keeler (1931), Tallila *et al.* (2008), Taulman *et al.* (2001), Tobin and Beales (2007)
used as a model for **ADPKD** OMIM 173900	***unknown***			*n/a*	n/a	Han:SPRD-Cy *(rat)*	Olbrich *et al.* (2003), Omran *et al.* (2001), Tobin and Beales (2008), Tobin *et al.* (2008)
bpk used as model for human **ARPKD** OMIM 263200 *jcpk* used as model for human **ADPKD** OMIM 173900	***BICC1***			*10q21.1*	**Protein bicaudal C homolog 1**	*bpk* *jcpk*	Torres *et al.* (2004), Tran *et al.* (2008), Tsiokas *et al.* (1997), Upadhya *et al.* (1999)

used as model for human **ARPKD** OMIM 263200	***CYS1***			*2p25.1*	**Cystin-1** Cilia-associated protein	*cpk*	Nauta *et al.* (1993), Nonaka *et al.* (2002), Upadhya *et al.* (2000), Utsch *et al.* (2006)
no Human syndrome	***KIF3A***			*5q31* ******604683*	**Kinesin-like protein KIF3A** Microtubule plus end-directed kinesin motor 3A	KIF3A null and conditional alleles	Kimberling *et al.* (1993), Koyama *et al.*(2007), Onuchic *et al.* (2002), Valente *et al.* (2003, 2005, 2006), Vierkotten *et al.* (2007), Vogler *et al.* (1999), Ward *et al.* (2002, 2003), Watanabe *et al.* (2003), Watnick and Germino (2003), White *et al.* (2007), Whitehead *et al.* (1999), Wilson *et al.* (2006)
no Human syndrome	***IFT52***				**Intraflagellar transport protein 52 homolog**	*Ift52^{hypo}*	Wolf *et al.* (2007), Wong *et al.* (2003)
no Human syndrome	***IFT88***	*TG737*	Used as model for human **ARPKD** OMIM #263200	*13q12.1* ******600595*	**Intraflagellar transport protein 88 homolog** Recessive polycystic kidney disease protein Tg737 homolog	*Orpk/polaris fxo*	Janaswami *et al.* (1997), Koyama *et al.* (2007), Marszalek *et al.* (2000), Morgan *et al.* (2002, 2003), Nauta *et al.* (1993), Nauli *et al.* (2003),

(*continued*)

Table 5.2 (*continued*)

Human Syndrome	HUMAN Gene	*also known as*	Associated Ciliopathy	Locus and OMIM	Protein	Murine model	Key References
							Nishimura *et al.* (2001), White *et al.* (2008), Wong *et al.* (2000, 2003), Wu *et al.* (1997, 1998), Xiong *et al.* (2002), Yodey *et al.* (1995, 2002)
no Human syndrome	***NEK1***		used as a model for human **ADPKD** OMIM 173900	*4q33* **604588*	**Serine/threonine-protein kinase Nek1** NimA-related protein kinase 1	*kat* *kat*^{2J}	Collin *et al.* (2002), Yodey *et al.* (2002); Zhang *et al.* (2001, 2002, 2003).

polydactyly is often seen in human BBS patients but has not yet been recorded in mutant mice. However, in other ways these models have been informative for clinicians; for instance, us and others recently reported anosmia in *Bbs1* and *Bbs4* mutant mice, which arises from a depletion of olfactory proteins in the ciliary layer of olfactory neurons (Kulaga *et al.*, 2004) and the presence of anosmia in human BBS patients, which is now established as a novel feature of the syndrome (Iannaccone *et al.*, 2005; Kulaga *et al.*, 2004).

Recently a study of craniofacial dysmorphology in BBS patients utilized no fewer than three organisms to delineate its underlying cause. Using three-dimensional (3D) facial imaging combined with dense surface morphometry and principle component analysis, Tobin *et al.* (2008) characterized the predominantly midfacial defects in BBS patients and demonstrated that these were also present in *Bbs4* and *Bbs6* mouse mutants. On the basis that human, mouse, and fish share similar embryonic craniofacial developmental processes and that most of the anterior head is derived from migrating cranial neural crest cells (NCC), zebrafish were used to demonstrate aberrant NCC migration when BBS genes are abrogated (Tobin and Beales, 2007; Tobin *et al.*, 2008). This study provides a quintessential example of the power of diverse model integration, something that is increasingly becoming commonplace within biomedical research.

3. Alström Syndrome

Alström syndrome (ALMS) is a rare recessive disorder that shows strong resemblance to BBS. It is characterized by cardinal features such as cone-rod dystrophy, neurosensory hearing loss, early-onset obesity, and insulin resistance leading to type 2 diabetes. A number of other features may also be present such as dilated cardiomyopathy, hepatic and urinary dysfunction, short stature, and male hypogonadism (Joy *et al.*, 2007). It has been demonstrated that variable sensorineural hearing loss in ALMS patients results from cochlear neuronal degeneration (Marshall *et al.*, 2005). Commonly, infants display nystagmus and photophobia, progressing to cone and rod photoreceptor degeneration, which gives a key differential diagnosis from BBS (Marshall *et al.*, 2005; Russell-Eggitt *et al.*, 1998).

Mutations in *ALMS1* account for all cases of this syndrome; again, there appears to be no phenotype–genotype correlations (Collin *et al.*, 2002; Hearn *et al.*, 2002). ALMS1 is ubiquitously expressed throughout all organ tissues (Collin *et al.*, 2002); it was first identified in a proteomic study of the centrosome (Andersen *et al.*, 2003) where it is localized to the centrosome and basal body (Hearn *et al.*, 2005), which suggested an involvement in ciliary function and a possible explanation for the phenotypic overlap seen with BBS (Hearn *et al.*, 2002). Although there are several conserved

sequence motifs within ALMS1, albeit of limited functional significance, its predicted sequence displays no similarity to any other known gene/protein. Studies in *foz* (*fat aussie*) mutants that carry a mutation in *Alms1*, as well as an *Alms1* knockout mouse, suggest that ALMS1 has a role in intracellular trafficking (Arsov *et al.*, 2006; Collin *et al.*, 2005). These mice develop features similar to ALMS patients such as obesity, hypogonadism, hyperinsulinemia, retinal degeneration, late-onset hearing loss and males, like in BBS null mice, are infertile due to aflagellate spermatozoa making this mouse an important model for further investigating disease manifestation in patients.

4. Polycystic Kidney Disease

The most commonly inherited PKDs are transmitted as autosomal-dominant (ADPKD) or autosomal-recessive (ARPKD) traits (Ibraghimov-Beskrovnaya and Bukanov, 2008). Although both forms of PKD initiate early in life, ARPKD rapidly progresses to kidney failure shortly after birth whereas ADPKD can take many years to reach end-stage renal disease (ESRD); typically by the fifth decade. Although both forms of the disease are characterized by the development and expansion of numerous fluid-filled cysts in the kidney as well as defects in multiple other tissues, the distribution of the renal cysts and the other body organs affected vary. Significantly, ADPKD is one of the most common human monogenic diseases with an incidence of 1:1000 and accounting for up to 10% of all end-stage renal patients; it is a systemic disorder, characterized by fluid-filled cysts not only in the kidneys but also in liver, pancreas, and other organs as well as cardiovascular defects and aneurysm. Although the incidence of ARPKD is lower at 1:20,000 it is associated with a high level of mortality in affected newborns. Most cases manifest *in utero* or at birth with renal enlargement and biliary dysgenesis. A number of well-characterized murine models, many spontaneous mutants, have been utilized to study the pathology of PKD (Guay-Woodford, 2003). By the mid-1990s, the genes mutated in the different types of human PKD were identified using positional cloning strategies. In the case of ADPKD, the causative genes are *PKD1* and *PKD2* that encode polycystin-1 and polycystin-2, respectively (Consortium, 1994; Mochizuki *et al.*, 1996), whereas mutations in a single gene, *PKHD1*, is sufficient to cause ARPKD (Onuchic *et al.*, 2002; Ward *et al.*, 2002; Xiong *et al.*, 2002) (Guay-Woodford, 2003). Subsequently, it was established that the disease-causing mutations in the murine models were not allelic to the genes known to cause PKD in humans. Additionally, there have been a number of reports demonstrating the profound effects of the genetic background of these animals on the disease phenotype (Gattone *et al.*, 1996;

Janaswami *et al.*, 1997). Despite this, these models have provided much insight into disease mechanisms and they have been instrumental in revealing a role for cilia in the mechanisms of cystogenesis; this will be discussed later.

5. Nephronophthisis

Nephronophthisis (NPHP) is an autosomal-recessive cystic renal condition characterized by corticomedullary clustering of cysts and tubulointerstitial fibrosis. In contrast to PKD where enlarged kidneys are a common diagnostic feature, the overall size of the kidney in NPHP is normal or diminished. Although, strictly speaking, NPHP describes a renal histopathology, ~10% of cases also present with extrarenal manifestations that can be associated with other syndromes such as retinitis pigmentosa (SLSN), cerebellar vermis hypoplasia (JBTS), ocular motor apraxia (Cogan type), cognitive impairment, hepatic fibrosis, phalangeal cone-shaped epiphyses (Mainzer-Saldino), and *situs inversus*. NPHP has also been described in cases of BBS, EVC, JATD, AS, and MKS (Hildebrandt and Zhou, 2007). NPHP presents as three forms characterized by the time of onset of ESRD: infantile, juvenile, and adolescent. Collectively, they constitute the most frequent genetic cause of end-stage renal failure in the young. The earliest presenting features are polyuria and polydipsia accompanied by renal concentrating defects. Eight causative genes have now been identified (summarized in Table 5.2) and the analysis of their protein products has provided a strong link between ciliary function and the pathogenesis of this disease. The time of disease onset is dependent on the variant form of NPHP involved such that *NPHP1*, *3–9* give rise to the juvenile and adolescent forms and *NPHP2* is responsible for the more severe infantile form. *NPHP1* encodes nephrocystin-1 a protein that interacts with other syndrome-related proteins: NPHP2, NPHP3, and NPHP4. Nephrocystin-1 localizes to adherens junctions and interacts with focal adhesion proteins within the renal epithelia with a likely role in cell polarity (Benzing *et al.*, 2001; Donaldson *et al.*, 2002; Nurnberger *et al.*, 2002). *NPHP2* was identified as the human orthologue of the murine *Inv* (*inversin*) mutant that encodes for the Inversin protein. Inversin interacts with β-tubulin, thus linking it closely to cilium structures (Otto, 2003; Watnick and Germino, 2003). *NPHP3* seems to be responsible for an adolescent form of the disease. The *pcy* (*polycystic kidney disease*) mouse was first described by (Takahashi *et al.*, 1986) and, at that time, identified as a model for ADPKD (Nagao *et al.*, 1991; Takahashi *et al.*, 1991). However, it was later revealed that the *pcy* mutation generated a hypomorphic allele for *Nphp3*, which itself is a cause of nephronophthisis in humans (Olbrich *et al.*, 2003; Omran *et al.*, 2001); this animal provided the first example of synteny between a human and a

spontaneous murine renal cystic disease. *NPHP4* encodes nephroretinin, which forms a complex with cell adhesion and actin cytoskeletal proteins (Mollet *et al.*, 2005). Mutations in *NPHP5/IQCB1* are associated with SLSN and NPHP5 interacts directly with RPGR (retinitis pigmentosa GTPase regulator) encoded by an X-linked gene which when mutated causes retinitis pigmentosa in males. Both localize to the connecting cilium of the photoreceptor and primary cilium of renal epithelial cells (Otto *et al.*, 2005). Perhaps the most important gene with respect to unifying the ciliopathies is *NPHP6/Cep290*. Cep290 is a novel centrosomal protein, the deficiency of which causes NPHP (type 6), JBTS (type 5), MKS (type 4), and BBS (type 14) (Valente *et al.*, 2006b). *Nphp6* knockdown in zebrafish leads to convergent extension defects, pronephric cysts, retinal degeneration, and hindbrain defects, nicely recapitulating the human disease (Sayer *et al.*, 2006). Furthermore, from the same study, *Nphp6* appears to regulate cell size and morphogenesis in the tunicate (sea squirt), *Ciona intestinalis*. *NPHP6* mutations cause Leber's congenital amaurosis (that affects the retina only) in 20% of patients diagnosed with NPHP (Chang *et al.*, 2006), which is supported by the finding of an in-frame deletion of *Nphp6/Cep290* in a *rds16* (*retinal degeneration*, *slow*) mouse, which is a model for retinitis pigmentosa (RP). Although the severity varies, RP is associated with mutations in most NPHP genes, except NPHP7. Mutations in *NPHP7/GLIS2* have only been reported in a single Oji-Cree Canadian family with disease confined to the kidney (Attanasio *et al.*, 2007). In support of this is the *Glis2* mutant mouse that develops, from 2 months postnatal, renal atrophy with fibrosis and increased apoptosis in renal tubular cells. Mutations in the novel *NPHP8/RPGRIP1L* gene causes MKS (type 5) and JBTS (type 7). Two truncating mutations were reported by Delous *et al.* (2007) in MKS fetuses whereas their JBTS patients carried missense and/or one truncating mutation, perhaps revealing a phenotypic spectrum of severity based on number and type of mutation. RPGRIP1L interacts with and colocalizes at the basal bodies, centrosomes, and primary cilia in renal tubular cells with nephrocystin-4 and nephrocystin-6 (Arts *et al.*, 2007). *RPGRIP1L* missense mutations were demonstrated to decrease the protein interaction with nephrocystin-4. *Rpgrip1l* is also one of the genes deleted in the (*Ft*) *fused-toe* mouse where the gene is referred to as (*Ftm*) *Fantom*. Mutations in *Ftm* have been linked to the renal and *situs inversus* phenotype in the *Ft* mouse. Rpgrip1l has recently been shown to participate in Sonic Hedgehog (Shh) signaling through the cilium thereby affecting patterning of the developing neural tube and limb (Vierkotten *et al.*, 2007). Intriguingly, further extrarenal manifestations have been reported in patients carrying *RPGRIP1L* mutations such as liver fibrosis, postaxial polydactyly, pituitary agenesis, and partial growth hormone deficiency (Wolf *et al.*, 2007), making this gene a plausible candidate for the rare RHYNS syndrome (retinitis pigmentosa, hypopituitarism, and skeletal

Table 5.3 Genetic and allelic heterogeneity of nephronophthisis (NPHP) and overlap with other ciliopathies, including: BBS – Bardet-Biedl syndrome; MKS: Meckel syndrome; JBTS: Joubert syndrome; NPHP: Nephrophthisis; SLSN: Senior-Løken syndrome.

Gene	NPHP	JBTS	MKS	BBS	SLSN
NPHP1	NPHP1	JBTS4			SLSN1
INVS	NPHP2				
NPHP3	NPHP3				SLSN3
NPHP4	NPHP4				SLSN4
IQCB1	NPHP5				SLSN5
CEP290	NPHP6	JBTS5	MKS4	BBS14	SLSN6
GLIS2	NPHP7				
RPGRIP1L/Ftm	NPHP8	JBTS7	MKS5		
NEK8	NPHP9				

dysplasia). Furthermore, the analysis of *jck* (*juvenile cystic kidney*) mice that carry mutations in *Nek8* (*never in mitosis gene A-related kinase 8*) led to the evaluation of *NEK8* in 588 NPHP patients revealing three missense mutations linked to a ninth NPHP locus (*NPHP9*) (Liu *et al.*, 2002). One patient had an additional mutation in the *NPHP5* gene raising the possibility that oligenic inheritance may be operating in some cases of NPHP. Nephronophthisis offers a clear example of the genetic and allelic heterogeneity that can be features of the ciliopathies; this is illustrated in Table 5.3.

6. Meckel Syndrome

The lethal autosomal-recessive Meckel syndrome (MKS) is characterized by renal cystic dysplasia with fibrotic changes in the liver, occipital encephalocoele or some other central nervous system malformation. Additionally, polydactyly is frequently reported and some patients have cleft palate, cardiac abnormalities, and incomplete development of genitalia and gonads (Dawe *et al.*, 2007; Paavola *et al.*, 1997; Salonen, 1984; Salonen and Paavola, 1998). Patients with MKS invariably die from respiratory and renal failure. Thus far, MKS has been linked to six loci of which five genes have so far been identified: MKS1, MKS3–5 (summarized in Table 5.2). All identified gene products are associated with ciliary functions. For instance, the products of *MKS1* and *MKS3/TMEM67* (meckelin) interact with each other and are required for centriole migration to the apical membrane and consequent formation of the primary cilium (Dawe *et al.*, 2007). *MKS3/TMEM67* has now been mapped to a region syntenic to the spontaneous *wpk* (*wistar polycystic kidneys*) locus in rat, which has polycystic kidney disease

(similar to ARPKD), agenesis of the corpus callosum, and hydrocephalus (Nauta *et al.*, 2000; Smith *et al.*, 2006). As described above, mutations in NPHP genes have been found in patients with MKS as well as JBTS suggesting that these conditions represent a spectrum of the same underlying disorder; these include *MKS4/CEP290/NPHP6* and *MKS5/RPGRIP1L/NPHP8*. Recently, a sixth locus for MKS was identified and the gene *MKS6/CC2D2*A reported; (Nagase, 2000) although the biological function of CC2D2A is uncharacterized, the identification of a calcium-binding domain as well as the fact that patient-derived fibroblasts lack cilia indicate a critical role for CC2D2A in cilia function (Noor *et al.*, 2008; Tallila *et al.*, 2008).

7. Joubert Syndrome

Joubert syndrome (JBTS) is an autosomal-recessive condition characterized by hypotonia, ataxia, severe psychomotor delay, oculomotor apraxia, and episodes of rapid breathing. Diagnosis may be supported by the neuroradiological hallmark referred to as the ''molar tooth sign'' (MTS), owing to horizontally oriented and thickened superior cerebellar peduncles and a deepened interpeduncular fossa combined with cerebellar vermis hypoplasia (Louie and Gleeson, 2005). The MTS has greatly enhanced the diagnosis of JBTS and with this has identified a group of Joubert syndrome-related disorders (JSRD) with involvement of other organs. For instance, approximately a quarter of patients develop juvenile nephronophthisis and retinal dystrophy, termed cerebello-oculo-renal syndrome (CORS) or Joubert syndrome type B (Valente *et al.*, 2003). Several additional clinical features have been reported including occipital encephalocele, polymicrogyria, cystic kidneys, polydactyly, hepatic fibrosis, and ocular coloboma, thus overlapping with the lethal, recessive disorder MKS and SLSN. Six of the eight JBTS loci have now been described (summarized in Table 5.2); again a clear overlap with other ciliopathies is apparent. The underlying gene mutations for *JBTS1* and *JBTS2* loci are not yet identified; however, *JBTS3* has been shown to be caused by mutations in the *AHI1* gene (Dixon-Salazar *et al.*, 2004; Ferland *et al.*, 2004) that accounts for between 7% and 11% of JBTS cases most of which are accompanied by retinopathy (Parisi *et al.*, 2006; Utsch *et al.*, 2006; Valente *et al.*, 2006a). The *AHI1* gene product is Jouberin; it is expressed in the brain and kidney and has been shown to interact with nephrocystin-1. In a mouse kidney cell line, AHI1 was shown to localize at adherens junctions, the primary cilia and basal body, which is consistent with a role in nephronophthisis (Baala 2007; Eley *et al.*, 2008). *JBTS6/TMEM67/MKS3* was identified as the sixth locus and linked to MKS. JBTS patients with associations with NPHP have mutations in *JBTS4/NPHP1*, *JBTS5/*

NPHP6/CEP290, and *JBTS7/NPHP8/RPGRIP1L* genes; the presence of RP and its severity is variable in these cases. *JBTS8/ARL13B* mutations have recently been identified in families with a classical form of JBTS (Cantagrel *et al.*, 2008). ARL13B belongs to the Ras-GTPase family and in other species is required for ciliogenesis, body axis formation, and renal function. Two animal models have been reported for this gene: the lethal *hnn* (*hennin*) mouse that has a coupled defect in cilia structure and Shh signaling and the zebrafish *scorpion* mutant that displays renal cysts and a curved tail, both of which are phenotypes common in morphants with cilia dysregulation (Cantagrel *et al.*, 2008; Caspary *et al.*, 2007; Garcia-Garcia *et al.*, 2005; Sun *et al.*, 2004).

8. Jeune Syndrome

Jeune asphyxiating thoracic dystrophy (JATD) is an autosomal-recessive chondrodysplasia. Affected children often die in the perinatal period owing to respiratory insufficiency that is a consequence of narrow and slender ribs and abnormal cage formation. Radiographical analysis can also indicate a shortening of the long bones and changes of the pelvic bones and the phalanges. There can be multiorgan involvement such as biliary dysgenesis with portal fibrosis and bile duct proliferation, renal cystogenesis and failure, polydactyly, and retinal degeneration.

These phenotypic clues led to the identification of mutations in *IFT80* in a subgroup of patients presenting with milder disease with no renal, liver, pancreatic, or retinal features (Beales *et al.*, 2007). There is, however, genetic heterogeneity with another, as yet unidentified locus on 15q. IFT80 is a member of the intraflagellar transport (IFT) complex B proteins that are important for cilia structure and function and which will be discussed further below. *Ift80* knockdown in the multiciliate protozoan, *Tetrahymena*, resulted in fewer cilia and nuclear duplication (Beales *et al.*, 2007). In *zebrafish*, silencing *ift80* results in convergent extension defects, cystic pronephros, and cardiac edema whereas knockout mice all display early embryonic lethality (unpublished observations).

9. Oral–Facial–Digital Syndrome

Oral–facial–digital (OFD) type 1 syndrome is an X-linked-dominant disease characterized by malformations of oral cavity, face, and digits and by cystic kidneys. Facial features include hypertelorism, broad nasal bridge, buccal frenula, cleft palate, lobulated tongue, lingual hamartomas; in the hands and feet, brachydactyly, and polydactyly may be present. PKD is

common and central nervous system malformations include corpus callosum agenesis, cerebellar abnormalities, and hydrocephalus, with accompanying mental retardation. It is presumed lethal in males. Mutations in the novel gene *OFD1* are causative and the OFD1 protein localizes both to the primary cilium and to the nucleus (Ferrante *et al.*, 2001; Giorgio *et al.*, 2007). *OFD1* has phenotypic similarities with 11 other forms of OFD syndrome, however, the underlying genes for these other disease forms remain to be identified. Franco and colleagues knocked out *Ofd1* in mice and recapitulated the human phenotype albeit with increased severity, possibly owing to differences of X inactivation patterns between species (Ferrante *et al.*, 2006). They also showed a failure of left–right axis specification in mutant male embryos, a lack of cilia in the embryonic node, mispatterning of the neural tube, and altered expression of *Hox* genes in the limb buds, all of which are indicative of cilia defects and demonstrate that Ofd1 plays a role in ciliogenesis.

10. The Structure and Function of the Cilium

Historically, much of what we know of the structure and workings of the cilium/flagellum has come from a number of model organisms whose strengths are include both that they are amenable to genetic manipulation and relatively simple organisms, compared to mammalian models making them ideal for microscopic analysis. These include the unicellular green algae flagellate, *Chlamydomonas reinhardtii*, the ciliated protozoan, *Tetrahymena*, and the nematode worm, *Caenorhabditis elegans*. The finding that the cilium and flagellum are structures that are evolutionarily conserved throughout nature means that what we learn from our more simple experimental models can be transferred to vertebrates, indeed humans. This has led to a rapid increase in our understanding of the biology and the consequences to development and disease when cilia are defective.

10.1. The structure of primary cilia

Cilia typically project from the apical surface of cells and are composed of a microtubular (MT) backbone (axoneme), nucleating from the basal body and ending at the tip complex, ensheathed by membrane contiguous with the plasma membrane. This generic structure is illustrated in Fig. 5.2; it indicates that the two types of cilia, motile and nonmotile, differ slightly in their structure. In general, motile cilia consist of an axoneme of nine microtubule doublets arranged in a ring with a central doublet pair (termed

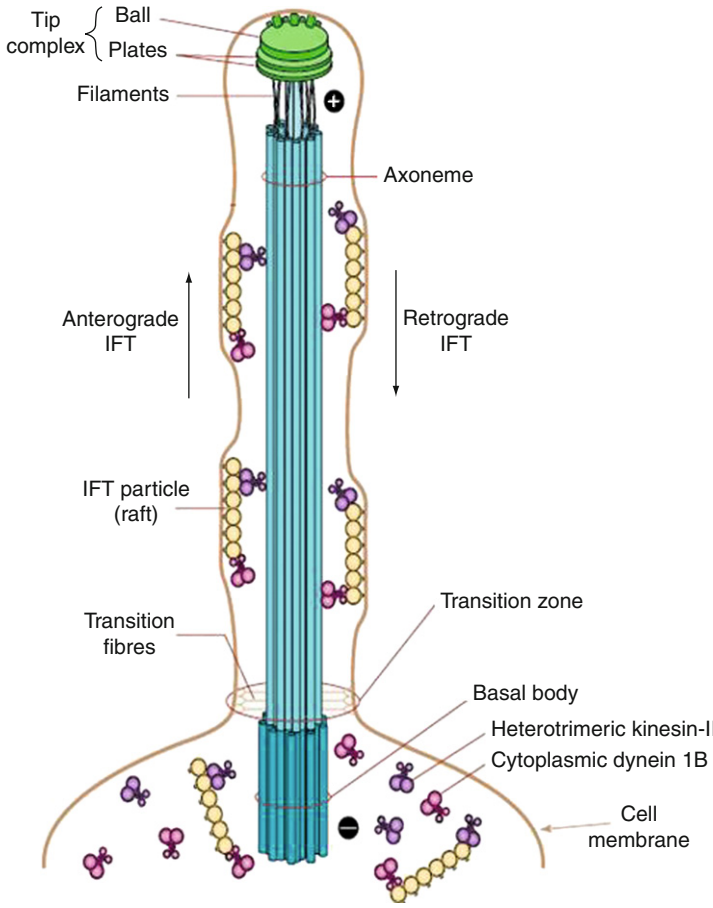

Figure 5.2 Structure of the cilium illustrating intraflagellar transport. Adapted from Eley *et al.* (2005) .

"9 + 2"). They tend to form in multiples on the cell surface and beat in concert with each other to provide unidirectional fluid flow. Each MT doublet consists of an "A" strand comprising 13-tubulin protofilaments, and a "B" strand made from 10 protofilaments (Alberts *et al.*, 2008). In motile 9 + 2 cilia, dynein motor proteins crosslink and enable doublets to slide against each other (Johnson and Gilbert, 1995; Porter and Sale, 2000). The outer and inner doublets are connected by radial spokes that serve to convert the sliding motion of the MT into a rhythmical beat; however, it is unknown how this beating is coordinated amongst cells. Nonmotile or "primary" cilia have a simpler 9 + 0 microtubule configuration, lacking the central doublet pair, dynein arms, and radial spokes. They are usually present as single apical membrane extensions.

10.2. Intraflagellar transport

Intracellular transport makes use of cytoskeletal microtubules and molecular motors to move membrane-bound vesicular cargo around the cell. Within the eukaryotic cilium/flagellum, cargo is also carried along the microtubular axoneme, in association with nonmembrane-bound protein complexes, in a highly conserved process termed, intraflagellar transport (IFT). As no protein synthesis apparatus exists within the cilium, one function of the IFT particle, is to carry cargo from the cell body, such as receptors and structural proteins into the cilia, as well as to deliver signals that emanate from the cilia into the cytoplasm in response to external environmental stimuli. Cargo is first translocated to a "loading dock" at the base of the cilium, which consists of transitional fibers that extend out from the basal body to the cell membrane (Deane *et al.*, 2001). Here a molecular motor–cargo complex is assembled and takes up its position at the proximal end (minus-end) of the axoneme in preparation for transport. The anterograde movement of the complex is facilitated by the heterotrimeric kinesin-II complex (Kif3a, Kif3b, and KAP) along the microtubules. At the tip (plus-end), the complex is remodeled and kinesin itself becomes cargo for retrograde transport back down the axoneme. This is accomplished by another motor, IFT-dynein (Pazour *et al.*, 1999; Porter *et al.*, 1999; Signor *et al.*, 1999). This switching of transport direction takes place only at the tip and basal body (Kozminski *et al.*, 1993). Particles undergoing IFT were first visualized on the axonemes of mutant *Chlamydomonas* with paralyzed flagella (Kozminski *et al.*, 1993, 1995). Interestingly, disruption of dynein-dependent retrograde transport can result in swelling of the cilium tip owing presumably to backup of cargo and highlighting the importance of this intricate but coordinated transport. Studies, in which IFT particles were isolated from *Chlamydomonas* flagella, identified a complex of 15 (now 17) polypeptides (Cole *et al.*, 1998; Piperno and Mead, 1997). This assembly was further resolved into two subcomplexes, A (550 kDa) and B (710–760 kDa) (Cole *et al.*, 1998). Sequence identity of these polypeptides revealed they are homologous to proteins required for sensory cilia assembly in *C. elegans* (Cole *et al.*, 1998; Perkins *et al.*, 1986; Starich *et al.*, 1995).

10.3. IFT and ciliopathies

The cilium is a highly regulated organelle, projecting from the surface of the cell, its membrane continuous with the plasma membrane of the cell and with a transport system that moves cargo between it and the cytoplasm of the cell. Evidence indicates that many proteins are seen to localize to the cilium. Hence the cilium can be described as a functional compartment, not only of the cytoplasm, but also of the plasma membrane itself with IFT

acting as a coordinated link between them. Studies into the pathogenesis of BBS in a number of different models have provided evidence for a functional link between the cilium and ciliopathies.

Most BBS proteins studied thus far localize to the cilium/basal body/centrosome complex. In mammalian cultured cells BBS1, BBS4, BBS5, BBS6, and BBS8 localize to the basal body and pericentriolar region whereas BBS4 and BBS5 have been observed in the ciliary axoneme (Ansley *et al.*, 2003; Kim *et al.*, 2004, 2005; Li *et al.*, 2004; Ross *et al.*, 2005) (and unpublished data). In *C. elegans*, BBS1::GFP, BBS7::GFP, and BBS8::GFP fusion proteins have been localized to the cilia and mutations for BBS7 and BBS8 show defective chemotaxis, behavior dependent upon functional ciliated neurons; thus, not only are the proteins localized to the cilia but are important for their proper function. Indeed, time-lapse microscopy studies in worms have shown that GFP::BBS7 and BBS8 fusion proteins, localized at the base of the cilium in ciliated neurons, are participating in IFT (Blacque and Leroux, 2006). This is further supported by evidence in *bbs7* and *bbs8* mutant mice where GFP-tagged IFT proteins fail to localize and move properly along the cilium suggesting that these proteins facilitate the incorporation of the IFT particle into the motor protein complex and are selective for particular cargoes. As indicated in Table 5.1, a common feature of the ciliopathies is a number of retinal pathologies. In mammalian photoreceptors, Rhodopsin relies on IFT for transport to the outer segment (a specialized cilia structure of the retina) and a feature of mouse *Bbs1*, *Bbs2*, *Bbs4*, and *Bbs6* mutants is the development of severe retinal degeneration akin to BBS patients (Fath *et al.*, 2005; Kulaga *et al.*, 2004; Mykytyn *et al.*, 2004; Nishimura *et al.*, 2004; Ross *et al.*, 2005). In these mouse mutants, degeneration is linked to failed Rhodopsin transport that subsequently accumulates in the cell body and triggers apoptosis (Nishimura *et al.*, 2004; Ross *et al.*, 2005). The development of a similar retinal degeneration in IFT mouse mutants supports the relationship between BBS and IFT (Pazour *et al.*, 2002a). It was later determined in *C. elegans* that BBS7 and BBS8 coordinate the movement of IFT subcomplexes A and B (Blacque *et al.*, 2004). In the absence of BBS proteins, the A and B subcomplexes move separately and at different rates, most likely because each is moved by a different type of kinesin. The authors proposed a model whereby BBS7 and BBS8 act as a bridge between the two IFT subcomplexes, stabilizing and coordinating transport, which may explain why BBS phenotypes are less severe than IFT knockouts. Figure 5.3 shows how BBS7 and BBS8 act together to coordinate IFT in *C. elegans*. Further evidence for the role of BBS proteins in IFT in mammalian cells comes from two-hybrid screens in which $p150^{glued}$ (a component of dynactin and important in retrograde motor function) and PCM1 (pericentriolar material 1) were identified as proteins that interact with BBS4. Silencing of *Bbs4* results in the abrogation of PCM1 recruitment to the pericentriolar

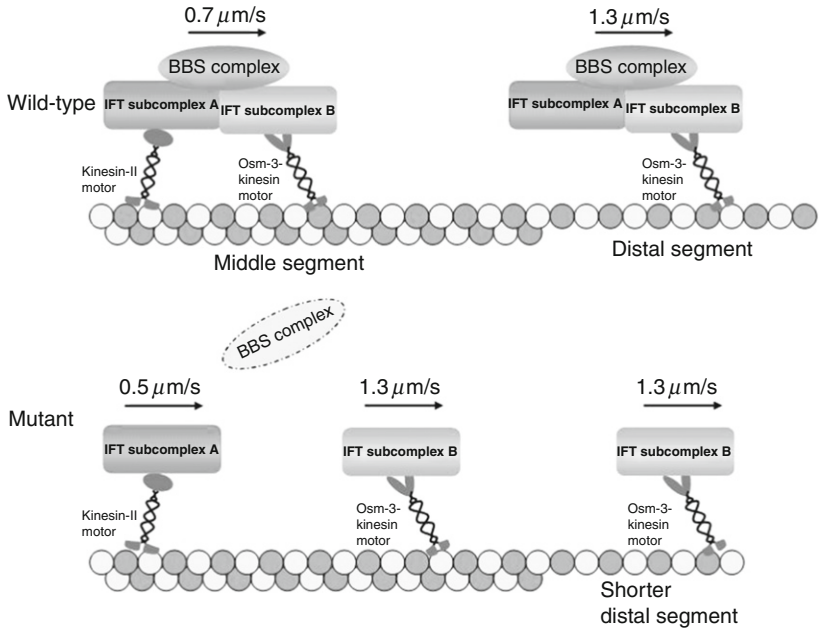

Figure 5.3 BBS7 and BBS8 act together to coordinate IFT in *C. elegans*. Upper panel. In wild-type worms, BBS proteins are transported along the ciliary midsegment by kinesin-II and OSM-3-kinesin at an "intermediate" rate (<0.7 μm/s). They travel faster (<1.3 μm/s) when reaching the distal segments propelled by OSM-3-kinesin alone. Lower panel. Loss of BBS protein function causes the separation of both kinesin-II motors and IFT particle subcomplexes A and B, resulting in subcomplex A moving at a slow rate (<0.5 μm/s) with kinesin-II, and subcomplex B moving at a fast rate (<1.3 μm/s) with OSM-3-kinesin. Redrawn with permission from Blacque and Leroux (2006).

satellites, deanchoring of microtubules from the centrosome, nuclear duplication, and blocked cytokinesis and apoptosis (Kim *et al.*, 2004). Thus, it has been proposed that BBS4 functions as an adaptor protein that is important for loading cargo (e.g., PCM1) onto IFT particles in preparation for its transport along the axoneme. Figure 5.4 illustrates how BBS4 modulates retrograde IFT.

Further insight into how BBS proteins behave, at the molecular level, was recently provided by (Nachury and Colleagues, 2007). Tandem affinity purification (TAP) has demonstrated that the BBS proteins form a complex termed the "BBSome." This 438 kDa complex consists of stoichiometric ratios of BBS1, 2, 4, 5, 7, 8, and 9. BBS9 is observed to interact with all other subunits and is therefore thought to act as a central organizer of the BBSome. The most marked defects in ciliation are found when cells are depleted of BBS1 and BBS5. One theory suggests that the BBSome is transported to the basal body by the centriolar satellites where it associates

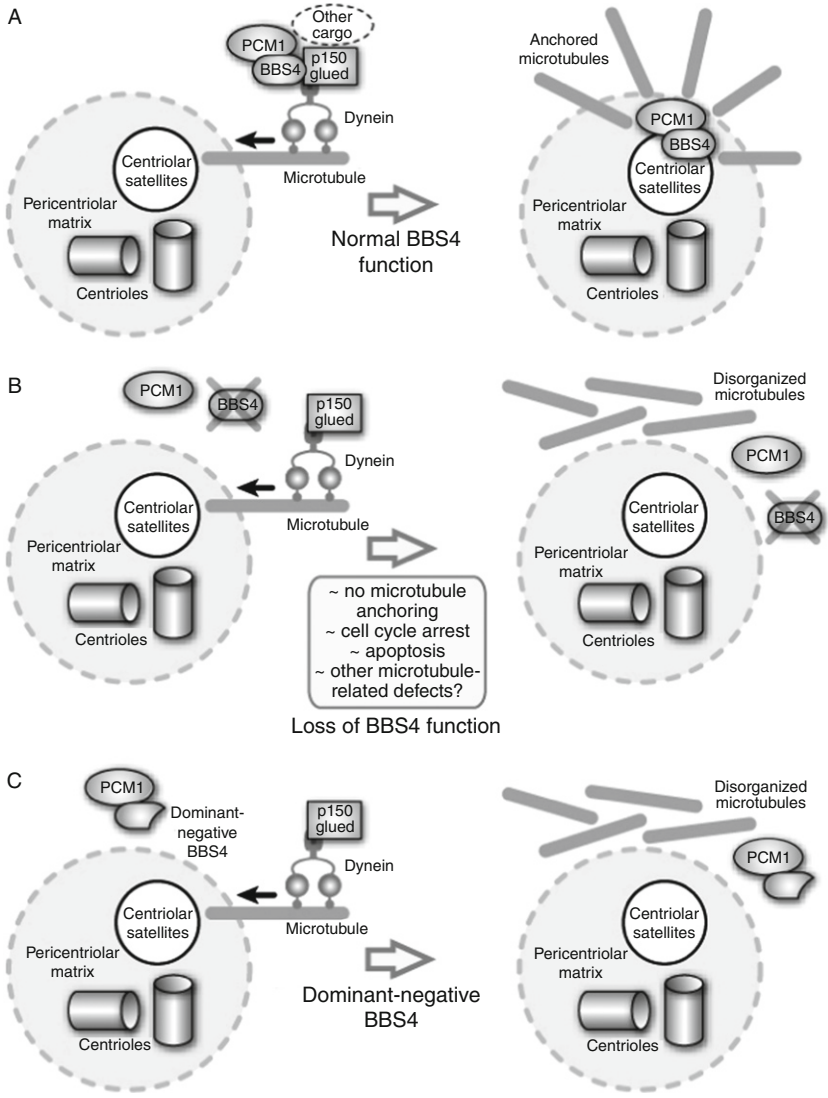

Figure 5.4 Model of BBS4 (dys)function. (A) In normal BBS4 function, BBS4 recruits PCM1 and associated cargo to the centriolar satellites in a dynein-dependent manner, likely through its interaction with $p150^{glued}$. (B) In the absence of BBS4, PCM1 and possibly its cargo do not localize to the centriolar satellites, leading to several cellular phenotypes. (C) Some BBS4 truncation mutants have an equally detrimental effect by inhibiting PCM1 localization to the centriolar satellites. Reproduced from Kim *et al.* (2004).

with the ciliary membrane. In addition, the BBSome interacts with Rabin8, a guanosyl exchange factor for Rab8 that is a small GTPase involved in regulating trafficking from post-Golgi vesicles. Rab8 itself enters the ciliary

membrane and promotes ciliogenesis, therefore, it has been proposed that pathogenesis in BBS might be caused by defects in vesicular transport to the cilium.

These data sets generate two general, nonexclusive models for how BBS proteins might function in relation to IFT. One suggests that at least BBS4, 7, and 8 are necessary for microtubule-based transport along the cilium, the other that BBS proteins function as a complex mediating vesicle transport from the cytoplasm to the cilium. As such, no one clear model for the function of BBS proteins has been described and the proteins may function in numerous roles, including cytoplasmic transport.

11. Cilia and Development

11.1. Left–right determination

A fundamental question perplexing developmental biologists for decades concerned the mechanism that establishes LR asymmetry, almost universal amongst vertebrates. A failure to break symmetry during early development can lead to a randomization of subsequent body organs, referred to as *situs inversus*, which is a common ciliopathy phenotype. LR asymmetry was first associated with cilia motility following observations of patients with a form of primary ciliary dyskinesia (PCD) known as Kartagener's syndrome that display increased pulmonary infections, male sterility, absence of sinuses/sinusitis and of a left–right organ reversal (Afzelius, 1976). Such disorders are associated with motile cilia dysfunction. Additionally, Afzelius accurately predicted that motile cilia are also important for cardiac situs during early embryogenesis. Since then mutations in genes encoding dynein heavy and light chains in the ciliary axoneme are responsible for some cases of Kartagener's syndrome (Giusto and Sciubba, 2004). Whilst studying the morphogenesis of the mouse embryonic notochordal plate as well as the node, situated at the rostral end of the primitive streak, Sulik *et al.* (1994) remarked upon the presence of a single, central cilium on each cell. Although they observed that cilia of the node and of the prechordal/notochordal plates were motile, they were unaware of the "potential significance of this motile behavior." It was later, in a landmark study, that Nonaka *et al.* (1998) demonstrated a clockwise rotation in the cilia lining the embryonic node that generated a leftward flow across the embryonic pole. Subsequent studies in the mouse mutant, *iv* (inversus viscerum) which results from mutations in the *lrd* (*left–right dynein*) gene showed a number of laterality defects as a result of abnormal nodal cilia and consequently fluid flow disruption (Okada *et al.*, 1999; Supp *et al.*, 1999). In fact, microfluidic experiments in which the fluid flow is reversed in the developing node produced *situs inversus totalis* in wild-type embryos thus establishing the

"morphogen flow" hypothesis (Nonaka *et al.*, 2002). This model proposes that the net leftward flow establishes a concentration gradient of signaling molecules such as Nodal, Fgf, or Shh across the node resulting in asymmetric gene expression and preferential activation of left-sided downstream pathways. Although there is undoubtedly leftward fluid flow, no left-sided accumulation of signaling proteins has been demonstrated. To account for this, a second hypothesis, or rather modification of the first has been proposed. The "two cilia" model is supported by the observation that two populations of primary cilia are found in the node (McGrath *et al.*, 2003). It is proposed that a centrally placed "rotating" population of cilia are surrounded by a population of nonmotile sensory cilia that "reads" the leftward flow by differential deflection and subsequent calcium influx and signaling. In support of this hypothesis, *lrd* mutant mice in which nodal cilia are paralyzed do not display the expected left-sided nodal calcium signal (McGrath *et al.*, 2003). Calcium signaling is likely triggered in response to cilia deflection during urine flow and it is known that it depends on polycystin-2, a major cause of adult PKD when mutated. Thus it is remarkable that *Pkd2* mice develop *situs inversus* in the presence of morphologically normal and motile nodal cilia (Pennekamp *et al.*, 2002). Consistent with the overall flow hypothesis are reports of cilia structural defects and laterality disorders in mouse mutants of the kinesin-II complex proteins (*Kif3a* and *Kif3b*) as well as *Invs*, *polaris* (*Tg737/Ift88*), *wimple* (*Ift172*), and *Rfx3* (Bonnafe *et al.*, 2004; Marszalek *et al.*, 1999; Mochizuki *et al.*, 1998; Murcia *et al.*, 2000; Nonaka *et al.*, 1998; Pennekamp *et al.*, 2002; Rana *et al.*, 2004) and in zebrafish embryos in which similar proteins such as *polaris*, *invs*, *lrd*, or *pc2* are abrogated (Bisgrove *et al.*, 2005; Essner *et al.*, 2005; Kramer-Zucker *et al.*, 2005b; Otto *et al.*, 2003; Sun *et al.*, 2004).

11.2. Hedgehog signaling in ciliopathies

An exciting development of the last 5 years has been the finding that cilia and IFT are essential for the activity of the HH pathway (Fig. 5.5) (Eggenschwiler and Anderson, 2007). HH signaling has a role in the development of nearly every organ in the body. Certainly, a role for cilia in the transduction of this pathway provides a valuable insight into mechanisms that may be underlying certain phenotypes common to the ciliopathies. In particular, we highlight phenotypes that arise in tissue where Shh signaling is known to play an important role during embryonic development in mammals; phenotypes such as postaxial polydactyly, external genitalia anomalies, and craniofacial defects.

Arguably, the best-known ligand for the HH pathway is Shh (Ingham, 2008). In addition to Shh, there are other Hedgehog ligand subgroups in vertebrates, such as Desert Hedgehog (Dhh) and Indian Hedgehog (Ihh). The downstream components of the pathway are the same, however, the

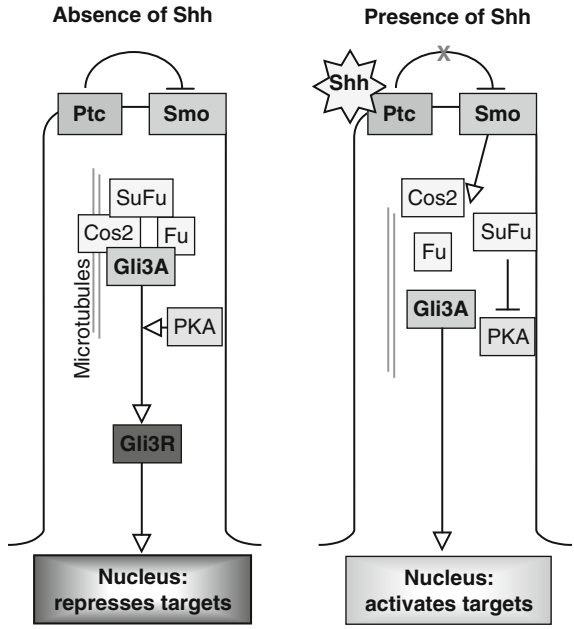

Figure 5.5 Mechanism of Shh transduction. In the absence of Shh, Ptc inhibits Smo. In the cytoplasm, the tetrameric complex of Su(Fu), Costal2, Fused, and Gli3 associates with microtubules and Gli3 is cleaved into its repressor form by PKA. In the presence of Shh, the inhibition of Smo by Ptc is relieved. Smo acts to disassemble the tetrameric complex, preventing the action of PKA and leaving active Gli3 free to enter the nucleus to induce target genes.

posttranslational modifications that effect diffusion kinetics vary between the different ligands types. Activation of the pathway relies on the export and extracellular diffusion of its ligand, which in the case of Shh is a cleaved 20 kDa N-terminal isoform called Shh-N, that binds to its membrane-bound receptor, Patched-1 (Ptc1). In the absence of bound ligand, Ptc1 inhibits Smoothened (Smo), a member of the G protein-coupled receptor family. Binding of Shh to Ptc1 relieves this inhibition on Smo, allowing the downstream transduction of the pathway signal that results in the transcriptional activation of target genes by the transcription factor, Gli3. A fundamental aspect of the pathway is that varying concentrations of Shh generate a graded response through the differential transcription of target genes. This morphogenic effect of Shh is encoded by a gradient of Gli transcriptional activity; in this way, Gli activity can be seen as the intracellular correlate of the extracellular Shh concentrations (Stamataki *et al.*, 2005). Hence, differential cell fate is determined by varying levels of Shh signaling, which in turn is dependent on the distance of the responding cells from the original source of secreted Shh. Ptc1 is an early response gene of the pathway, which acts itself to restrict the diffusion of the ligand.

The first link between IFT and the HH pathway was reported in embryonic patterning mutants from an ethyl nitrosourea (ENU)-based mutagenesis screen. Huangfu *et al.* (2003) identified two IFT mutants, called *wim* (*Ift172*) and *flexo* (*Ift88*) (refer Table 5.2); although these embryos expressed Shh normally, their *Ptc1* expression levels are markedly down-regulated indicative of a loss of pathway activity. Further analysis in the neural tube showed that ventral cell types are absent while dorsal cell types, usually repressed by Shh, are expanded. Cilia are present on neural tube cells and the cilia in these embryos are absent or stunted. Subsequently, many of the downstream components of the pathway, at some stage in their activity, show subcellular localization to the cilium. These include Smo, Ptc1, and Gli2/Gli3, as well as a number of proteins that interact with Gli in a tetrameric complex that regulates the proteolytic conversion from an active isoform (Gli3A) to a repressor isoform (Gli3R); this includes Suppressor of Fused (Su(Fu), a negative regulator of the pathway and Costal2 (Cos2), a microtubule-associated kinesin-like protein. (Corbit *et al.*, 2005; Haycraft *et al.*, 2005; Ocbina and Anderson, 2008; Rohatgi *et al.*, 2007). Given these insights, it is perhaps unsurprising that IFT plays an essential role in Shh signaling (Haycraft *et al.*, 2005; May *et al.*, 2005). Several other ciliary proteins have since been shown to be essential for Shh signaling, including THM1 and RPGRIP1L/Ftm/JBTS7/MKS5 (Tran *et al.*, 2008; Vierkotten *et al.*, 2007).

In the case of embryonic limb bud development, Shh is known to play an important role in anteroposterior patterning. Shh ligand itself originates from a source at the posterior margin of the limb bud in a region called the zone of polarizing activity (ZPA). Ihh is expressed in chondrocytes and acts to signal to the growth plate to induce cell division essential for bone growth. Interestingly, mutations in *IHH* in humans are associated with bone growth defects such as brachydactyly (short fingers) and dysplasia of the long bones such as the femur (Hellemans *et al.*, 2003). Loss-of-function mutations to *SHH* results in a loss of digits, while mutations in *GLI3* cause polydactyly (McGlinn and Tabin, 2006), a common feature of ciliopathies in general and in BBS, present in at least 70% of patients (Tobin and Beales, 2007). It is interesting, as mentioned above, that polydactyly is not, however, a feature of mouse BBS mutants.

Beales *et al.* (1999) have reported that 98% of BBS patients have malformed external genitalia. In males this includes micropenis, small and undescended testes, and hypospadias (mispositioning of the urethral meatus). This is recapitulated in *Shh* null mice that fail to grow out a genital tubercle, the precursor to the penis (Haraguchi *et al.*, 2001). Perriton *et al.* (2002) showed that transplanting the normal urethral epithelium to the limb bud in chick embryos was sufficient to induce mirror-image duplication of the digits, implying that Shh is acting to also pattern genital development. Additionally, Dhh is expressed in the testis and involved in spermatogenesis.

Thus, the genital and limb phenotypes seen in ciliopathies, such as in BBS and MKS, may share an etiology underpinned by aberrant Shh signaling. In support of this hypothesis, it is interesting to note that ALMS is often misdiagnosed as BBS; patients present similar features: obesity, blindness, deafness, and kidney disease. However, ALMS patients do not develop polydactyly or genital anomalies, features likely attributable, at least in part, to faulty Shh signaling.

In the skull, Shh signaling is essential for normal craniofacial development; in particular it is essential for development of the frontonasal and maxillary processes of the upper and midface (Hu and Helms, 1999). Mutations in *Shh* in mice and humans cause holoprosencephaly (HPE) where the lobes of the forebrain fail to split, leading to a single central eye (cyclopia) and the absence of a nose, but with a less severe effect in the lower face. During embryogenesis, Shh is expressed in a rostral region of the forebrain as well as within the oral ectoderm and it has been demonstrated that these sources of Shh are important for the correct migration of cranial NCC that contribute to the bony features of the face (Eberhart *et al.*, 2006). Interestingly, craniofacial dysmorphology is a notable feature of BBS and it is likely that this may reflect, in part, defects in Shh signaling (Tobin *et al.*, 2008). It is noteworthy that homozygous mutations in *Rab23* have been shown to cause Carpenter syndrome characterized by craniosynostosis, polysyndactyly, obesity, and cardiac defects. (Jenkins *et al.*, 2007) Rab23 is a small GTPase involved in the endocytosis or targeting of vesicles containing Shh, Ptc, or Smo (Eggenschwiler *et al.*, 2006).

11.3. Cilia and polarity

Cells within an epithelium often display a polarity across the plane of cells known as epithelial PCP. Recently, PCP genes have also been shown to have a role in ciliogenesis and, conversely, genes previously known to be required for ciliogenesis have a role in PCP (Jones and Chen, 2007). Examples of PCP in vertebrates include the uniform orientation of stereociliary bundles on the apical surfaces of sensory hair cells in the organ of Corti of the mammalian auditory sensory organ (Kelly and Chen, 2007), as well as convergent extension (CE) movements that involve a polarized cellular rearrangement that leads to the narrowing and concomitant lengthening of a tissue along two perpendicular axes (Jessen *et al.*, 2002), both linked to cilia. Vertebrate PCP signaling utilizes some of the components of the canonical Wnt/β-catenin signaling pathway. Wnt signaling is most well known for its role in embryogenesis and cancer but is also important for a number of normal physiological processes in adult animals. The central purpose of the Wnt/β-catenin pathway is to stabilize β-catenin by preventing its phosphorylation-dependent degradation. This involves the binding of extracellular Wnt ligands to receptors of the Frizzled (Fz) and

LRP families on the cell surface causing the activation of Disheveled (Dsh or Dvl) family proteins. Dsh inhibits a second complex of proteins, Axin, GSK3, and APC, which normally promotes the proteolytic destruction of β-catenin. Consequently, the inhibition of the "β-catenin destruction complex" affects the amount of β-catenin that reaches the nucleus to interact with TCF/LEF family transcription factors to promote specific gene expression.

Vertebrate PCP signaling also requires the binding of Wnt ligand to its Fz receptor. Because extracellular ligands and membrane receptors constitute the first signaling modalities in a pathway, it is often tempting to think that specific Wnt ligand and/or ligand/receptor combinations dictates the strict spatiotemporal specificity of pathway activation, as well as the outcome of downstream signaling events. However, many examples of ambiguity are now emerging that caution taking too simplistic a view. Additionally, the subcellular localization of Dsh is also now known to have an important impact on the switch between the different branches of the pathway (Wnt/β-catenin or PCP). In PCP signaling, Dsh localizes to the plasma membrane via its DEP domain where it is activated by Fz in combination with a coreceptor, Knypek (Wong *et al.*, 2000, 2003). Dsh activation does not involve β-catenin or the destruction complex in PCP signaling but instead involves a number of proteins specific to PCP signaling. For instance, the nonclassical cadherins Fat, Dachsous and Flamingo, and other proteins, including Prickle and Strabismus act downstream of Fz and Dsh to regulate the cytoskeleton through RhoA and Rho-kinase (Habas *et al.*, 2003). Figure 5.6 outlines the main steps in canonical Wnt and PCP pathways.

Konig and Hausen (1993) were the first to implicate cilia in planar polarity events, although at the time they did not know that this involved PCP signaling. They described the polarity of epithelial cells in the embryos of the African clawed frog, *Xenopus laevis*, aligned with the direction of ciliary beating. Cano *et al.* (2004) subsequently demonstrated that the subcellular localization of β-catenin was altered in *Ift88* mutant mice and that this affected the organization of pancreatic tissue. Perhaps the most significant finding to link PCP signaling to the cilium and consequently to ciliopathies, was that of (Simons and colleagues 2005) that showed Inversin (NPHP2) localizes to the cilium and acts as a molecular switch between the Wnt/β-catenin and PCP branches of the pathway. This is achieved through the degradation of cytoplasmic pools of Dsh, in contrast to membrane-bound Dsh that is linked to PCP signaling (Simons *et al.*, 2005). Interestingly, fluid flow such as the flow of urine in the developing renal tubules, may act to increase the level of Inversin in cultured kidney cells, which has been speculated to favor the patterning of renal epithelia over cellular proliferation. In ciliopathy mutants, where PCP signaling is suppressed, the balance of proliferation and differentiation appears to be lost, leading to cyst formation. Further evidence to support the role of PCP signaling in

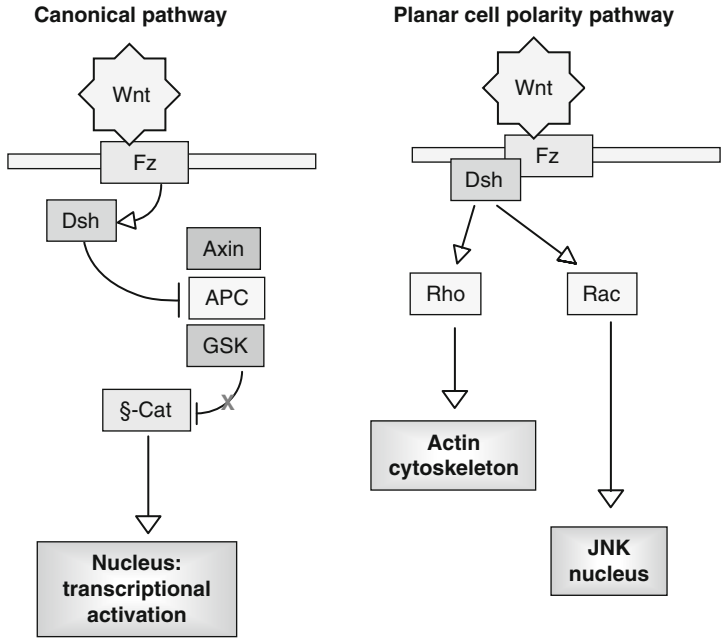

Figure 5.6 Wnt signaling pathways. In the canonical pathway, Wnt binds to its receptor Frizzled (Fz) which activates cytoplasmic Disheveled (Dsh). This inhibits the destruction complex consisting of Axin, APC, and GSK3β, preventing the destruction of cytoplasmic β-catenin. β-catenin then enters the nucleus and activates transcriptional targets by association with TCF and LEF. In the PCP pathway, Wnt binds to Fz which recruits Dsh to the plasma membrane. This results in RhoA activation and consequently remodeling of the cytoskeleton. Dsh can also activate the JNK pathway, through Rac1, to affect the transcription of downstream targets of this pathway.

normal renal development comes from a mouse mutant for the PCP gene *Fat4*, which develops cystic kidneys as a result of disoriented cell divisions and tubule elongation (Saburi *et al.*, 2008).

Mouse PCP mutants such as *circle tail*, *loop tail*, *crash*, and *Dvl1* and *2*, display neural tube defects (NTD) such as spina bifida (failure of posterior neural tube closure), craniorachischisis (failure of the entire neural tube to close), and exencephaly (failure of rostral neural tube closure) (Doudney and Stanier, 2005). These gene knockout models tend to be embryonic or perinatal lethal. In humans, missense mutations in *VANGL1*, the mouse orthologue of the gene mutated in *loop tail*, causes NTD such as spina bifida (Kibar *et al.*, 2007). Ross and colleagues demonstrated that BBS proteins are involved in PCP signaling and that a proportion of *Bbs4* null mice phenocopied PCP mutants such as *loop tail* (Eley *et al.*, 2005). They had open eyelids at birth, exencephaly, and misoriented stereociliary bundles. Compound *Bbs4/Lt* heterozygotes recapitulated the phenotype, whereas single heterozygotes for each allele was not phenotypic, implying a genetic

interaction between the two genes. Vangl2, a membrane-bound receptor involved in PCP signaling, localizes to the cilium. Analysis of downstream effector proteins for the PCP pathway have demonstrated that mutations in *inturned* and *fuzzy* abrogate PCP signaling and cause indirect Shh-deficient phenotypes as a result of defective ciliogenesis (Park *et al.*, 2006). It is likely that affecting the structure and/or function of the cilia and its associated proteins acts to disrupt the balance between the different branches of the Wnt signaling pathway. Corbit *et al.* (2008) showed that the Wnt pathway components β-catenin and APC are also localized to the cilium. Additionally, *Ofd1* null cells that lack cilia are five times more sensitive to stimulation with exogenous Wnt ligand compared to wild-type cells, probably because the cilium in normal cells act to impose a brake on the Wnt/β-catenin pathway. In support of this, Gerdes *et al.* (2007) showed that suppressing expression of *bbs1*, *bbs4*, *bbs6*, and *Kif3a* affects the stabilization of β-catenin and consequently the upregulation of TCF-mediated transcriptional activity, which is indicative of overactive Wnt/β-catenin signaling.

11.4. Cilia and cystogenesis in mammals

Although *C. elegans* has no kidney *per se*, the worm has provided many seminal moments in renal-related research, beginning with the initial findings that *C. elegans* harbor orthologues of the human polycystins, known to be one of the primary causes of PKD in humans (Mochizuki *et al.*, 1996) and that these proteins (called LOV1 and PKD2) are found to be involved in mating behavioral responses mediated by sensory neurons that require cilia function (Barr and Sternberg, 1999). Subsequently, IFT88 was identified in algae and worms (where it is called Osm-5) and shown to be the underlying mutation in the important *orpk* (*oak ridge polycystic kidney*) mouse that models ARPKD (Moyer *et al.*, 1994). This work demonstrated that IFT is a conserved process common to all ciliated or flagellated eukaryotic cells and that the IFT protein, *Polaris/Tg373*, is important for ciliogenesis in mammals, thus, providing the first evidence of a link between cilia dysfunction and renal cyst formation (Haycraft *et al.*, 2001; Pazour *et al.*, 2000, 2002a; Qin *et al.*, 2001; Taulman *et al.*, 2001). In the mammalian kidney, primary cilia extend from the apical surface of renal epithelial cells into the tubule lumen as well as from cells of the nephron (with the exception of intercalated cells) and it is now known that proteins whose functions are disrupted in cystic diseases have all been localized to the cilium or the basal body. The model currently held for how primary cilia function in the renal epithelium is that they act as mechanosensors of extracellular fluid flow through the lumen of renal tubules to regulate cell growth and differentiation (Nauli *et al.*, 2003). Invariably, a failure in this balance between proliferation and differentiation seems to be the underlying cause of abnormal cell proliferation leading to the production of renal cysts (Lina and

Satlinb, 2004) and it seems that deflection of the axoneme due to flow is the initiator of an increase in intracellular calcium signaling that results in altered gene expression (Yoder, 2007). Polycystin-1 and polycystin-2 have been shown to interact with each other (Qian *et al.*, 1997; Tsiokas *et al.*, 1997) and are thought to be part of a calcium channel localized in the primary cilium of renal epithelial cells (Gonzalez-Perrett *et al.*, 2001; Hanaoka *et al.*, 2000; Pazour *et al.*, 2002b; Somlo and Ehrlich, 2001; Yoder *et al.*, 2002). Furthermore, *PKHD1* (the underlying cause of ARPKD) that encodes polyductin localizes to primary cilia and has been shown to affect renal duct differentiation (Onuchic *et al.*, 2002; Ward *et al.*, 2002, 2003; Xiong *et al.*, 2002). However, other findings show that polycystin-1 can regulate the expression of p21, a tumor suppressor that inhibits cyclin-dependent kinases (CDK) leading to cell-cycle arrest (Bhunia *et al.*, 2002) and that IFT88, required for cilia assembly, has an additional role in regulating cell-cycle progression that is independent of its function in ciliogenesis (Robert *et al.*, 2007). This highlights the need for a better understanding of the relationship between the cilium, cell-cycle, and cilia-mediated mechanosensation and signaling activity.

Certainly, there are now many well-characterized murine models for studying human renal cystic diseases. The hypomorphic nature of the *orpk* allele (originally named $Tg737^{orpk}$ but now designated as $Ift88^{Tg737Rpw}$) allows this animal to survive into young adulthood, making this a valuable model to study cystic renal phenotypes (Moyer *et al.*, 1994). The early onset of the renal phenotype and the subsequent distribution of cysts in the kidney has made this animal a good model for human ARPKD. *Chlamydomonas* mutants for Ift88 have a complete absence of flagella, the *orpk* mutants have stunted and malformed cilia, but are not completely abolished. Given the localization of IFT88 in the cilia (Haycraft *et al.*, 2001; Moyer *et al.*, 1994; Pazour *et al.*, 2000; Taulman *et al.*, 2001) and its requirement for ciliogenesis, it is not surprising that abnormal cilia are observed in the renal epithelium of *orpk* mice. The original gross phenotype of the *orpk* mouse described scruffy fur, severe growth retardation and preaxial polydactyly on all limbs (Moyer *et al.*, 1994). Subsequent work has revealed *orpk* mice to also have random LR axis specification, liver and pancreatic defects, hydrocephalus, and skeletal patterning abnormalities in addition to PKD. A role supported by the loss of cilia on the ependymal cell layer in brain ventricles and by the lack of node cilia in the Ift88 mutants (Taulman *et al.*, 2001). Additionally, the follicular dysplasia that gives rise to the disordered and scruffy fur may suggest a role for primary cilia in the skin and hair follicle and provide insight into ectodermal dysplasias seen in the human syndromes such as Ellis-van Creveld and Sensenbrenner's syndromes (Lehman *et al.*, 2008). Thus, this model continues to provide insights into the role of cilia in multiple tissues.

Mandell and colleagues first described the *cpk* (*congenital polycystic kidney*) mouse for which the underlying mutation was subsequently found to be in the *Cys1* gene (Hou *et al.*, 2002; Mandell *et al.*, 1983); the finding that its gene product, cystin, localizes to the cilium along with polycystins (Yoder *et al.*, 2002) means this mouse continues to be a useful model for studying a recessively transmitted form of PKD. The *jck* (*juvenile cystic kidneys*) mouse that carries a recessive mutation has an intermediate phenotype relative to *cpk* and *pcy* with mutants being fertile and surviving into adulthood (Atala *et al.*, 1993).

ADPKD has also been difficult to model. Although probably not an entirely satisfying theory, it has long been proposed that there is a two-hit mechanism required for cyst formation, consisting of a germ line mutation to one allele and a somatic mutation to the other (Ong and Harris, 2005). Embryonic renal cyst development in homozygous knockout *Pkd1* or *Pkd2* animals and the progressive cystic disease associated with the $Pkd2^{WS25}$ mutant, which has a *Pkd2* allele prone to inactivation by somatic mutation, are consistent with a ''two-hit model'' of cyst development (Lu *et al.*, 1997; Wu *et al.*, 1998). In all homozygous animals, renal development appears to be normal until embryonic day (E) 14.5 after which renal cysts start to develop, suggesting that the polycystins are not required for nephronic induction. But these mutations are generally embryonic lethal due to systemic effects. In contrast to the human condition, the phenotype seen in heterozygote mice is generally mild and variable probably due to the late onset of this disorder, reflecting the difference in lifespans between humans and mice. Transheterozygotes are somewhat more severe. This and a number of conditional knockouts of *Pkd1* that are emerging as orthologous models of ADPKD suggest a productive future for therapeutic testing (Lantinga-van Leeuwen *et al.*, 2004; Wu *et al.*, 1998).

A number of PKD models with an autosomal-dominant inheritance pattern are also available; for instance, the Han:SPRD rat has been well documented with several features which resemble human ADPKD (Cowley *et al.*, 1993; Gretz *et al.*, 1996) despite the fact that the disease in these animals is not linked to the rat orthologue of the human PKD1 gene (Nauta *et al.*, 1997).

Interestingly, the genetic background in these murine models has a strong influence on the expression of the mutated gene and consequently how comparable the disease manifests between mice/humans. For instance the *cpk* mouse has been extensively studied in the C57BL strain but it was only when the *cpk* gene was bred onto a CD1 background (Gattone *et al.*, 1996) that the extrarenal pathology, similar to that seen in the human infantile PKD, was penetrative. Additionally, Janaswami *et al.* (1997) reported that the genetic background had profound effects on the disease phenotype in the *kat*(*2j*) (*kidney*, *anemia*, *testes*) mouse. These observations have also been reported in most other murine models.

11.5. Cilia and obesity

Obesity is a cardinal feature of two ciliopathies whose diagnosis is often confused: BBS and ALMS. In BBS, the frequency of overweight varies by from 72% (Beales *et al.*, 1999) to 91% (Green *et al.*, 1989) whereas childhood obesity is present in 95% of ALMS patients (Joy *et al.*, 2007). Although obesity is a common thread for certain ciliopathies, its precise cause is not yet clear. Nevertheless, this does raise the question as to whether cilia function is required for body mass homeostasis. In BBS patients, it has been proposed that obesity arises from hyperphagia (overeating) and that this feeding behavior is perhaps a result of hypothalamic defects impacting on the satiety centre where ciliated neurons regulate hunger and satiation (Davenport *et al.*, 2007). Others have found that BBS patients tend to be less physically active, albeit with no significant difference in their energy metabolism compared to BMI matched controls (Grace *et al.*, 2003). In support of findings in human patients, mouse BBS mutants have been shown to consistently eat more than their wild-type littermates and go on to display early-onset obesity. Additionally, Rahmouni *et al.* (2008) found BBS2, 4, and 6 null mice have combination of low locomotor activity. These mice were also shown to have and increased levels of circulating leptin, a hormone that regulates satiety. Whereas the administration of exogenous leptin to wild-type mice reduced bodyweight by around 10% in a 4-day period, no such effect was seen in the BBS mutants, regardless of the underlying mutation. This suggests that hypothalamic neurons in the BBS mutants are unresponsive to leptin, which results in changes in their feeding behavior. Hypertension is a common feature of BBS patients; it is interesting that the mouse BBS mutants also develop hypertension most likely as a result of this resistance to leptin signaling. As mentioned above, the *foz* mutant mouse, which carries a mutation in Alms1, also develops hyperphagia and obesity as well as insulin tolerance progressing to type 2 diabetes (Arsov *et al.*, 2006). Although fibroblasts taken from ALMS patients do not indicate a role for ALMS1 in ciliogenesis, the protein itself localizes to the cilium and basal body (Hearn *et al.*, 2005). Other proteins involved in the regulation of feeding behavior also show a distribution to the cilium, such as the G protein-coupled receptor Melanin concentrating hormone receptor-1 (Mchr1), which is abrogated in BBS mutant mice (Berbari *et al.*, 2008). Davenport *et al.* (2007) further provided the first insight into a mechanism for obesity. They conditionally ablated Kif3a, therefore blocking ciliogenesis, in POMC (pro-opiomelanocortin) expressing hypothalamic neurons involved in negatively regulating appetite. These mice most notably display an increase in feeding behavior that progressively leads to obesity. These data have provided important evidence for a role of cilia in regulating appetite and indirectly suggests that BBS proteins and ALMS1 play a role in appetite homeostasis.

12. Therapies for Cystic Disease

Of all phenotypes seen within the ciliopathies, it is the renal pathologies that are the greatest contributor to morbidity and mortality in affected patients. As an example, in ADPKD, cyst growth can lead to dramatic increases in overall kidney mass; at times up to 20 kg (Germino and Somlo, 1993). Eventually, the size and number of cysts replace functional tissue and normal kidney filtration is impaired, resulting in ESRD in approximately 50% of patients. The only effective treatment currently available to PKD patients is transplantation, which emphasizes the need to develop potential therapeutic agents for clinical use. Because the mode of cystogenesis is probably similar irrespective of the gene mutated, there are opportunities for treatment using small molecules/drugs to treat the cystic diseases seen in the ciliopathies. A number of drugs are currently under investigation for their ability to slow cyst development and these represent significant hope as a treatment strategy (summarized in Table 5.4). Of particular mention are Rapamycin and Roscovitine, both of which have well-characterized modes of action, and here we will highlight findings from their use in cystic kidney models.

Rapamycin (Sirolimus) is an inhibitor of mTOR pathway that is an important regulator of cell proliferation; it is currently being used in clinical trials in humans. It has been proposed that the inappropriate activation of mTOR is a common feature of all forms of cystic kidney disease and may indeed be the predominant cause of PKD (Edelstein, 2008a,b). Interestingly, mTOR interacts with the cytoplasmic tail of polycystin-1, indicating that it functions, at least in part, within the cilium (Shillingford *et al.*, 2006). Certainly, in support of this, Rapamycin treatment alone is able to rescue kidney phenotypes in several different mouse models. For instance, Rapamycin treatment in mice with cystic kidneys increases apoptosis and shedding of cystic cells, resulting in an overall reduction in the size of the kidneys from 16% of total body weight to 4%, compared to 0.7% in wild-types (Shillingford *et al.*, 2006). In these mice, renal function, as measured by blood urea nitrogen concentration, was completely restored. Upstream of mTOR are two centrosomal proteins, TSC1 and TSC2 that, when mutated, cause the Tuberous sclerosis complex with renal cysts (Henske, 2005; Wilson *et al.*, 2006).

Roscovitine is also currently in clinical trials for cancer treatment. It is an inhibitor of cell cycle, acting through CDK. Cell-cycle regulation, disrupted in PKD through a ciliary mechanism, is a plausible target for preventing cyst growth (Ibraghimov-Beskrovnaya and Bukanov, 2008). Both in humans and in the *jck* mouse, an increase in proliferating cells lining the renal tubules has been reported, which is reduced with Roscovitine treatment (Bukanov *et al.*, 2006). The increased proliferation seen within diseased tubules is attributed to an upregulation in the activity of the Ras

Table 5.4 Drugs previously used on rodent models of polycystic kidney disease. [adapted from 213]

Treatment	Mode of action	Model used
Acetazolomide	Carbonic anhydrase inhibitor; diuretic	Han:SPRD rat (PKD model). Did not work in PCK rat
Amiloride	Diuretic	Han:SPRD rat. Did not work on PCK rat
Batimastat	Matrix metalloprotease inhibitor, anti-cancer drug	Han:SPRD rat
c-myc antisense	Prevents excess cell division	*Cpk mouse*
c-Src inhibitor	Prevents excess cell division	*Bpk* mouse (ARPKD model)
EGFR inhibitor	Inhibits receptor for epidermal growth factor	Han:SPRD rat
Lovastatin	HMG-CoA reductase, lowers cholesterol level	HAN:SPRD rat
Methylprednisolone	Corticosteroid, blocks inflammation	Han:SPRD rat and *pcy* mouse
Paclitaxel	Stabilises microtubules to prevent cell division. Used in cancer chemotherapy	*Cpk* mouse (ARPKD model). Did not work on *pcy* (*Nphp3*) mouse, *orpk* mouse, or Han:SPRD rat.
Rapamycin	Inhibits mTOR, antiproliferative compound	*Bpk* and *orpk* mice
Roscovitine	CDK-inhibitor; prevents cell division	*Jck* (*Nek8*) and *cpk* mouse
V2R antagonist	Vasopressin receptor antagonist	PCK rat, *cpk*, *pcy*, *Pkd2* mice

mitogenic signaling pathway through Erk2 (Bukanov *et al.*, 2006). Ras acts via Raf to translocate the MAP kinase, Erk, to the nucleus where it phosphorylates transcription factors associated with cell division. Additionally, Cdk7 and Cdk9 activities are also upregulated and these represent likely targets for Roscovitine. In Roscovitine treated *jck* mice, the overall size of the kidney is reduced from 9% to 4% of bodyweight with concomitant improvements in kidney function as measured by a decrease in blood urea nitrogen. *Nek8*, which is mutated in the *jck* mouse, localizes to the cilium where it interacts with polycystin-1 and polycystin-2 (Sohara *et al.*, 2008). In the more aggressive forms of PKD, such as the *cpk* mouse that is a model for ARPKD, the affect of Roscovitine have been less effective (Bukanov *et al.*, 2006). In terms of treatment strategies, it is interesting to note that treatment with Roscovitine for 3 weeks followed by 2 weeks without is sufficient to reduce cyst size, therefore, providing long lasting effects without a need for daily administration.

As discussed above, several important mouse mutants have been important models for studying the pathogenesis of PKD. These include *orpk* mouse, *polaris*, *inv*, *cpk*, and a conditional null allele for *Kif3a* that is expressed in the kidney (refer Table 5.2). Increasingly, zebrafish are becoming an attractive model for investigating human renal cystic diseases as they offer distinct advantages for ease in conducting high-throughput chemical or mutagenesis screens (Drummond, 2005). Zebrafish are also amenable to genetic manipulation and furthermore can absorb small molecules from the water making them desirable for drug screening protocols. Tobin and Beales (2008) recently reported a high-throughput and rapid method for drug testing in zebrafish using translation and splice-blocking morpholino oligonucleotides (MOs) to knock down a number of genes known to cause visible cystic lesions in the kidney: *bbs4*, *bbs6*, *bbs8* (BBS); *nphp2* (NPHP), *nphp5* (SLSN), *nphp6* (JBTS); *mks1*, *mks3* (MKS), *ofd1* (OFD). In these morphants, Rapamycin and Roscovitine were used to ameliorate the renal phenotype; in both cases the pronephric cysts were rescued, although Rapamycin demonstrated significantly better results over Roscovitine. Thus zebrafish provide a cost-effective approach to identify compounds for preclinical studies in mice and then man.

13. Concluding Remarks

Since the discovery of the cell by Robert Hooke (1663) and bacteria and protozoa by Anton van Leeuwenhoek (1673), the history of cell biology has been punctuated by epic advances. The cilium, that century-old object of curiosity, has recently enjoyed a rebirth, and with it has come astonishing biological revelations marking the next chapter in cellular history. Perhaps, unlike its forbearers, the rapidity of discoveries linked to

the cilia is equally as remarkable and we owe these fortunes to the development of animal models. Foremost amongst these is undoubtedly the house mouse, the research value of which has been acknowledged since the early 1900s (Keeler, 1931; Sage, 1981). It is the recognition that humans and mice share the same organ systems, similar reproductive cycles, skeletons, biochemistry, physiology, and most importantly pathologies that have enabled us to progress our understanding of the basis of human disease and this shows no sign of abating in the foreseeable future. It is important, however, that we recognize the limitations of the mouse as a model for human disease, most particularly where there are differences, albeit subtle at times, between human and mouse in both physiology and genetics. An example of this, in relation to the ciliopathies, is the lack of extra-digit formation in BBS mutant mice, despite its common appearance in other mutants and humans. We predict that polydactyly in BBS patients results from perturbation of SHH signaling; therefore, it is either the intricacies of the pathway that differ or the response thresholds that are dissimilar, or in fact that the background strains (all reported mouse mutants are mixtures of the two most common strains: C57/BL6 and Sv129) are harboring modifier genes of expression. Despite this, mouse models of the ciliopathies have been extraordinarily insightful, providing the opportunity to study mechanisms of renal cystogenesis, cell polarity, and tissue patterning. There is, however, currently a shortage of mouse models for the ciliopathies, many of which suffer from embryonic lethality necessitating the need for the generation of conditional mutants. In coming years we expect to see the development of more models, such as to investigate cardiac (e.g., *Cited2*) and brain malformations that are consequent upon ciliary perturbation. We also expect to witness an increase in the use of mutant mice for translational research.

The interpretation of disease models in other organisms equally comes with their benefits and pitfalls. Using renal cystic disease as an example, although mouse models develop renal cysts, it is often not until several months of age and these animals must be euthanized and sectioned to reveal the cysts. In contrast, zebrafish stands out as a relatively new model organism, which has been particularly successful for studying renal cystic kidney disease (Drummond, 2005). Among the key advantages of zebrafish over mammals is that it breeds quickly and often (daily). Its embryos develop outside the body and are transparent, making developmental defects (such as renal cysts) easy to observe, often without sectioning. Pronephric cyst formation in zebrafish is readily observable as a bubble appearing 3 days postfertilization (dpf). Drummond *et al.* (1998) exploited this observation in a large-scale mutagenesis screen from which they identified 15 cystic mutants. A later screen using retroviral mutagenesis identified 10 more cystic mutants, four of which encoded IFT proteins (Sun *et al.*, 2004).

The fish renal system is much less complex than that in mammals, reflecting its aquatic environment, comprising only two nephrons

(compared with 1 million in a mammalian kidney). Two glomeruli are fused in the midline from which the pronephric tubules emanate caudally. These glomeruli are anatomically similar to mammals containing fenestrated epithelia and podocytes for ultrafiltration. In contrast to land-dwellers, however, the pronephros is primarily designed for osmoregulation rather than excretion and significant insults to the system are poorly tolerated. Whilst the effects may be similar to PKD in humans, it is important to be mindful of the fact that the pathomechanisms may not be identical and the proof of principle findings now emerging in zebrafish will need to be taken back into mammalian models.

Although both mammalian and fish nephrons contain cilia, ultrastructurally these vary with mammals having 9 + 0 (nonmotile) and fish having 9 + 2 (motile). These motile cilia in fish beat with a frequency of about 20 Hz establishing a current along the tubule (Kramer-Zucker *et al.*, 2005a) and it has been suggested that a reduction in this ciliary motility results in the buildup of pressure and consequent cystogenesis. A contrasting study examined the mechanism of cyst formation in several zebrafish mutants and found that relatively early (48 hpf) tubule dilation precedes excess cell proliferation (Sullivan-Brown *et al.*, 2008). They also established that despite different degrees of dysmotility in each mutant, the mechanism and extent of cyst progression is the same, suggesting that cystogenesis is independent of cilia motility. A third study sheds further light on the possible mechanisms of cyst formation following the discovery of *seahorse*, a zebrafish mutant (Kishimoto *et al.*, 2008). Seahorse is not required for ciliogenesis or motility, yet the mutants still develop cysts. The authors discovered that Wnt/β-catenin signaling is ectopically induced in these mutant fish, at the expense of PCP signaling, and furthermore that seahorse interacts directly with Dsh, a key component of both pathways. This underscores the role of Wnt signaling (perhaps via the cilium) in cyst formation.

As the number of ciliopathies expands to take in more diverse diseases and organ systems, it is now becoming possible to predict putative disorders based on phenotyping (Badano *et al.*, 2006; Beales *et al.*, 2007). Conversely, a number of mutant gene models discussed here do not yet have human counterparts. It was assumed that in some cases, such as the Ift88/polaris mouse that given the embryonic lethality corresponding human mutations would not be viable. This view has now been challenged with the identification of mutations, albeit ''mild'' (missense and single codon in-frame deletion) in IFT80 as a cause of JATD (Beales *et al.*, 2007). We should now expect to find hypomorphic mutations in other IFT-related genes; the problem arises, however, in predicting the likely phenotypes. This should be possible using a reverse integrative genomics–phenomics approach whereby shared phenotypes likely share pathways or groups of proteins (Oti *et al.*, 2008). A ciliopathy example would be the Tubby mouse, which

has all the attributes of the human ALMS and some of the BBS but no mutations have yet been found.

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