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Role of the RNA-binding protein Bicaudal-C1 and interacting factors in cystic kidney diseases

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ABSTRACT

Polycystic kidneys frequently associate with mutations in individual components of cilia, basal bodies or centriolar satellites that perturb complex protein networks. In this review, we focus on the RNA-binding protein Bicaudal-C1 (BICC1) which was found mutated in renal cystic dysplasia, and on its interactions with the ankyrin repeat and sterile α motif (SAM)-containing proteins ANKS3 and ANKS6 and associated kinases and their partially overlapping ciliopathy phenotypes. After reviewing BICC1 homologs in model organisms and their functions in mRNA and cell metabolism during development and in renal tubules, we discuss recent insights from cell-based assays and from structure analysis of the SAM domains, and how SAM domain oligomerization might influence multivalent higher order complexes that are implicated in ciliary signal transduction.

1. Introduction

Polycystic kidney diseases (PKD) are rare chronic kidney diseases (CKD) that share several cancer hallmarks [1] and an elevated cancer risk, especially in kidneys [2]. PKD and the related ciliopathy nephronophthisis (NPHP) (literally "disappearance of nephrons") vary in aggressiveness and distribution of cysts and extrarenal symptoms: In NPHP-like ciliopathies, cysts may arise secondarily to interstitial fibrosis during or before adolescence due to autosomal recessive mutations in 1 or 2 out of > 90 genes [3]. Another aggressive early cystic disease called autosomal recessive PKD (ARPKD) mainly arises from mutations in fibrocystin. The slowest but more frequent form (1/2300) is autosomal dominant PKD (ADPKD). ADPKD results from heritable mutations in *Polycystic kidney disease 1 (PKD1)* or *2* (*PKD2)* genes that encode the transient receptor potential ion channel polycystin 1 (TRPP1) or 2 (TRPP2), respectively [4]. Complexes of TRPP1 and TRPP2 (here abbreviated PKD1 and PKD2) localize to the ER, cell surface and primary cilia to regulate Ca^{2+} currents, mTORC1 and other effector pathways in response to diverse stimuli [5]. Impaired Ca^{2+} flux increases cAMP and protein kinase A (PKA) signaling, altering its normal anti-proliferative output to growth stimulation [6,7]. In addition, *PKD1* mutations activate Rho-ROCK signaling, leading to ectopic nuclear YAP and elevated c-Myc expression that accelerate cystic growth [8]. Several key insights have come from studying PKD2 during visceral left-right patterning. During development, PKD2 localization in node cilia enables asymmetric Nodal signaling and organ patterning by downregulating the Nodal antagonist Dand5 on the left side [9]. PKD2 is activated by a directional flow of extracellular fluid. To generate flow, motile node cilia must rotate clockwise and coordinate their strokes by tilting their axes towards the posterior in a process governed by WNT/planar cell polarity (PCP) signals [9]. Available data point to intersections of these pathways with BICC1, ANKS3 and ANKS6 both during left-right patterning and in renal tubules. After describing known connections and some unresolved questions, we discuss how SAM domain proteins interact with each other and with other ciliopathy proteins. Although we only begin to appreciate the complexity of the multivalent interactions involved, the resulting protein networks emerge as dynamic RNA-binding platforms that may couple cell metabolism and post-transcriptional regulation of gene expression to cilia.

2. Developmental and kidney functions of BICC1 homologs

2.1. Conserved role in renal tubules

A link of BICC1 to cystic kidney diseases was discovered by positional cloning of the recessive *bpk* allele (Balb/c mice with Polycystic Kidneys), and of a chlorambucil-induced allele in juvenile congenital polycystic kidney (*jcpk*) mice [10–12]. In *bpk*, a frame-shift in exon 22 replaces the final 21 residues of Bicc1A, the major splice variant, by 149 aberrant amino acids (Fig. 1). By contrast, *jcpk* leads to more aggressive

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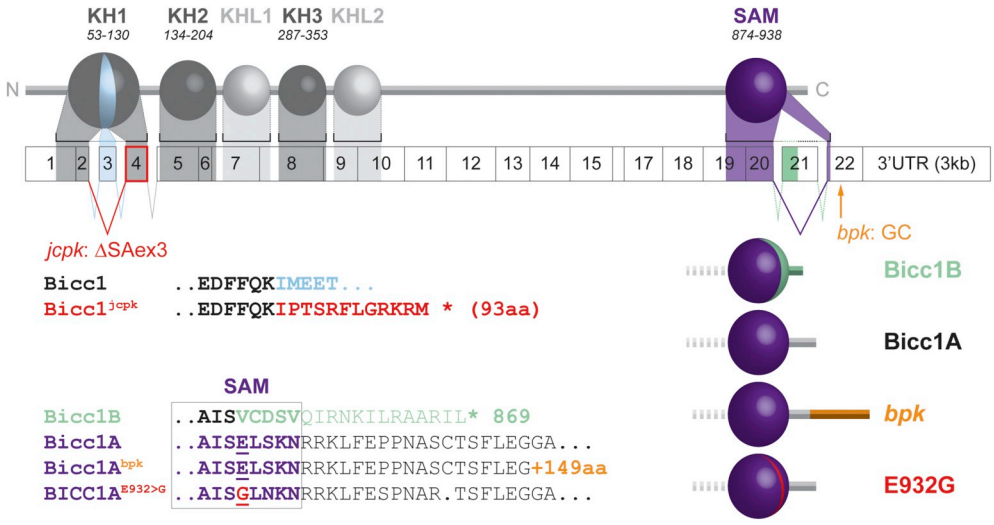

Fig. 1. BicC mutant alleles in mouse and human.
Positions of mutations, and the C-terminal sequences of the SAM domain of both splice variants in mouse and human are indicated. KH and KHL domain boundaries for the mouse sequence are as in [50].

disease with occasional cysts even in heterozygotes (Table 1) due to a frame shift and premature stop already after residue K81 in both splice variants. Human *BICC1* mutations have been reported in two pediatric cases with unilateral cystic renal dysplasia, including a heterozygous premature stop codon after Q87 reminiscent of *jcpk*, or the E932G mutation in the SAM domain of BICC1A [13]. *Bicc1* null mutants in addition show *situs* randomization, but heterozygotes remain asymptomatic [14–16]. (Fig. 1). Since neither *bpk* nor *jcpk* have been reported to develop *situs* defects, they are unlikely null alleles. Depletion of BICC1 homologs also induces *situs* alterations and pronephric cysts in *Xenopus* and zebrafish embryos [16,17], or cystic Malpighian tubules in *D. melanogaster*[18], suggesting a conserved role in renal tubule homeostasis.

2.2. Roles of BICC1 homologs during embryogenesis

Bicaudal-C (*bicc*) was discovered in *Drosophila* as a maternal effect gene with dominant double abdomen phenotypes resulting from premature mRNA translation of oskar (osk) in oocytes [19–21]. Anterior structures can be rescued by mutations in the cytoplasmic polyadenylation element-binding protein Orb, which normally is bound and inhibited by BicC, but poised to increase *osk* translation by recruiting poly(A) polymerase [22]. BicC consists of a tandem repeat of Heterogeneous Nuclear Ribonucleoprotein K homology (KH) and KH-like (KHL) domains at the N-terminus, separated from a C-terminal SAM domain by a serine- and glycine-rich intervening sequence. Consistent with a regulatory role in mRNA translation, BicC can bind poly(U) sepharose, and a stem-loop in Gurken (Grk) mRNA [21,23], and a missense mutation in a KHL domain de-represses Osk protein synthesis [20]. However, association with *osk* mRNA has not been demonstrated.

A single vertebrate BicC homolog designated Bicc1 (formerly xBicC) was first identified in *Xenopus*. It stimulates dorsal endoderm formation independently of its SAM domain, and this activity can be blocked by a dominant-negative mutant form lacking KH domains [24]. In sea urchin, depletion of maternal BicC inhibits both endoderm and anterior neurogenic ectoderm formation [25]. In mouse, *Bicc1* expression is first detected in node cells, and its loss results in misalignment of motile cilia, disruption of leftward flow and *situs* randomization [14]. An analogous phenotype is observed in *Xenopus* embryos after *bicc1* knockdown in left-right organizer cells, suggesting a conserved role in axis formation [14]. Since there are no other homologs, we generally refer to BicC, whereas the abbreviations Bicc1 and BICC1 distinguish mouse and human proteins.

2.3. Intersections with cyst-promoting pathways

Motile node cilia and their rotational axes are aligned by PCP signaling of Wnt receptor complexes with Dishevelled (DVL) at the plasma membrane (Fig. 2A) [9]. Cytoplasmic DVL can be sequestered by Inversin (INVS), which is mutated in NPHP2 patients [26], or by binding to Bicc1 [14] to avoid interference by canonical WNT/β-catenin signals (Fig. 2B). Another link to WNT was found in *Xenopus* where BicC represses maternal Wnt11b mRNA by binding its 3'UTR [27]. Although WNT/β-catenin signaling does not increase in Invs mutant cells [28], it accelerates PKD progression in *Pkd2* mutant mice [29]. Several WNTs also bind PKD1 to potentiate PKD2/Ca^{2+} signaling [30]. Western blot analysis of *Pkd1*$^{-/-}$ mouse kidneys and microarray profiling of ADPKD tissue revealed that PKD1 stimulates *Bicc1* expression [18,31], acting together with the transcription factor Hnf1β which (is essential to prevent cyst formation during development Fig. 2C) [32]. However, whether *Bicc1* expression depends on WNTs is unknown.

Analysis of *bpk* kidneys showed that Bicc1 negatively regulates EGFR and mTORC1 signaling [33–35]. In addition, *Bicc1* null mutant kidneys accumulate elevated levels of cAMP and adenylate cyclase 6 (Adcy6) [15]. Bicc1 is epistatic with PKD2: It increases *Pkd2* mRNA levels [16,36], and *Pkd2* mRNA injection partially rescued pronephros formation in *bicc1*-depleted *Xenopus* embryos. By contrast, injection of Bicc1 mRNA failed to rescue *pkd2* morphants [16]. Together, these observations place BicC downstream of PKD1 and primarily upstream of PKD2 (Fig. 2C). Whether PKD2 in addition stimulates BicC activity remains to be tested.

2.4. A new role for Bicc1 in renal gluconeogenesis

ADPKD progression is accelerated by metabolic reprogramming linked to mitochondrial dysfunction [37–42]. A proteomic screen for Bicc1-interacting factors in T-Rex HEK293 cells identified multiple proteins linked to cellular metabolism [43]. Among these, the C-Terminal to Lis-Homology domain (CTLH) complex was previously shown in *S. cerevisiae* to target the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and fructose-1,6-bisphosphatase 1 (FBP1) for degradation specifically in high glucose medium [44,45]. Bicc1$^{-/-}$ neonates develop severe hypoglycemia with impaired PEPCK and FBP1 expression in kidneys but not in liver. Bicc1 knockdown also

Table 1
Role of SAM domain proteins linked to cilia or cyst formation, and of interacting NIMA-related kinases

Gene	Function	SAM[a]	Ref.	year
BicC	• *bpk* and *jcpk* mouse models of PKD harbor mutations in *Bicc1* SAM or KH domains, respectively	+	[12]	2003
	o *bpk* homozygous Balb/c mice die 2-4 weeks after birth, with biliary tract hyperplasia and renal cysts in proximal tubules and collecting ducts.	+	[10]	1993
	o Modifier loci in C57Bl/6 accelerate PKD in *bpk* mice.		[106]	2000
	o *jcpk* mice die 7-10 days after birth, with cysts in all nephron segments, frequent gall bladder pancreatic or bile duct dilation. Glomerular cysts also form in 25% of aged heterozygotes.		[11]	1995
	• Targeted *Bicc1* knockout revealed an additional role in cilia polarization and visceral left-right patterning;		[14]	2009
	Left-right randomization of visceral *situs* in *Xenopus* morphants	n.d.	[16]	2010
	• Cystic pronephros and reduced pkd2 expression in *Xenopus* morphants	n.d.	[17]	2010
	• Zebrafish morphants develop cystic pronephros and slow urine flow	+	[13]	2012
	• Heterozygous nonsense and missense mutations in patients with unilateral cystic renal dysplasia	n.d.	[36]	2016
		n.d.	[18]	2017
	• Pancreatic duct dilation and increased immune infiltration in *Bicc1* knockout mice	n.d.	[43]	2018
	• *bicc* mutant flies develop cystic dilations of Malpighian tubules			
	• Metabolic re-wiring of *Bicc1* mutant kidneys includes inhibition of renal gluconeogenesis			
ANKS6	• Autosomal dominant R823W mutation in the SAM domain found in PKD/Mhm(cy/+) rat model of PKD	+	[66]	2005
		+	[107]	2010
	• Transgenic R823W *Anks6* triggers cysts in rat kidneys, but not in other tissues.	+	[108]	2014
	• R823W transgenic rats hyperactivate β-catenin			
	o c-Myc expression increases, while Cdkn1a levels decrease		[56,109]	2017
	o Transcription of the miR-17∼92 cluster by c-Myc may mediate Cdkn1a mRNA silencing.	n.d.	[76]	2013
	• Zebrafish morphant embryos develop pronephric cysts and cardiac *situs* inversions	n.d.		
	o *anks6* depletion in *Xenopus* epidermis cells mildly perturbs planar polarity of basal bodies	+	[76,108]	2013
	• Homozygous missense or splice mutations and truncations in NPHP patients	+	[68]	2015
	• ENU-induced I747N mutation in the SAM domain provokes cystic kidneys in mice		[69]	2015
	• M187K homozygotes in addition develop heterotaxia marked by right pulmonary isomerism			
ANKS3	• Antisense oligonucleotide injection increases vasopressin signaling (cAMP)	n.d.	72]	2015
	• Zebrafish morphants develop pronephric cysts and heart situs randomization	n.d.	[70]	2015
	o *anks3* promotes cilia polarization in the pronephros	n.d.		
	• Nearly complete *situs* inversion in H147N mutant patient	n.d.	[71	2016
	o Truncation of zebrafish anks3 after R272 by a C888 > T point mutation is embryonic lethal			
	• *Anks3* null mutant mice die in utero. The phenotype is not characterized (MGI:5763008)			
NEK8	• Autosomal recessive G442V mutation in juvenile cystic kidney (*jck*) mice	n.a.	[77]	2002
	• Comparison of *jck/jck* with *jck/-* mice revealed that G442V is a gain-of-function mutation	n.a.	[78]	2013
	• Homozygous and compound heterozygous mutations in human NPHP9 provoke sustained nuclear shuttling of YAP/TAZ, diminished retention of phospho-YAP in cilia, and genomic instability.	n.a.	[81,82,110]	2008
			[111,112]	2012
		n.a.	[78]	2013
	• Null mutant mice develop cardiac malformations and *right* pulmonary isomerism (inhibition of Nodal)	n.a.	[69]	2015
	• Mice homozygous for the kinase-dead I124T allele develop *left* pulmonary isomerism (ectopic Nodal)			
NEK7	• Knockout mice are growth retarded and mostly die in utero (mixced 129/ICR background)	n.a.	[96]	2010
	o Fewer fibroblasts are ciliated; centrosome multiplication; cytokinesis failure; polyploidy		[	
	o Survive for up to 4 weeks on Balb/c genetic background			
	• Required for centriole duplication	n.a.	[93]	2011
	• Suppresses ciliogenesis in proliferating cells during G1 interphase	n.a.	[95]	2017

n.d.: not determined; n.a.: not applicable.
[a] Functions that depend on the SAM domain.

diminished FBP1 protein in mIMCD3 and LLC-PK1 kidney cell lines, whereas Bicc1 overexpression increased it. However, rather than directly targeting FBP1, the CTLH complex diminished the levels of Bicc1 protein [43]. In conclusion, the mammalian CTLH complex does not directly control gluconeogenic enzymes, but instead targets Bicc1 (Fig. 2C).

3. Interactions with RNA

3.1. BicC target mRNAs

Microarray profiling identified 89 mRNAs enriched in BicC immunoprecipitates from *Drosophila* ovaries, including *bicc* and *dishevelled* transcripts [46]. In Malpighian tubules, BicC also co-immunoprecipitates d-myc mRNA [18]. The first mRNAs found to bind BicC in vertebrates emerged among candidate regulators of protein kinase A (PKA) signaling, including PKA inhibitor α and *Adcy6* transcripts, as well as Bicc1 mRNA itself [15]. Bicc1 protein levels vary along the nephron, and elevated Adcy6 protein levels correlate with excess cAMP accumulation in *Bicc1* mutant kidney extracts [15]. Sequencing of transcripts associating with HA-tagged BicC in *Xenopus* embryos identified the Nodal antagonist Coco (Dand5) and the co-receptor xCR1, among others [47]. Since BicC functions primarily in cilia positioning and flow regulation [14], testing a role downstream of cilia in the Nodal pathway remained challenging. Adding to this complexity, BicC also binds mRNA encoding DDX5, a DEAD-box ATP-dependent RNA helicase implicated in regulating multiple aspects of RNA metabolism and WNT signaling [48].

KH domains contact RNA via conserved GXXG motifs, where at least one X is arginine or lysine. Binding to GXXG orients four single-stranded nucleotides towards a groove of hydrophobic and electrostatic determinants for nucleobase recognition [49]. KH-like domains lacking GXXG motifs do not bind RNA. A crystal structure of tandem KH domains of the BicC-related protein GLD-3 suggests that all RNA-binding clefts congregate in a common surface (Fig. 3A) [50,51]. Binding to the xCR1 3'UTR is mediated mainly by the KH2 domain via an ACAUUA

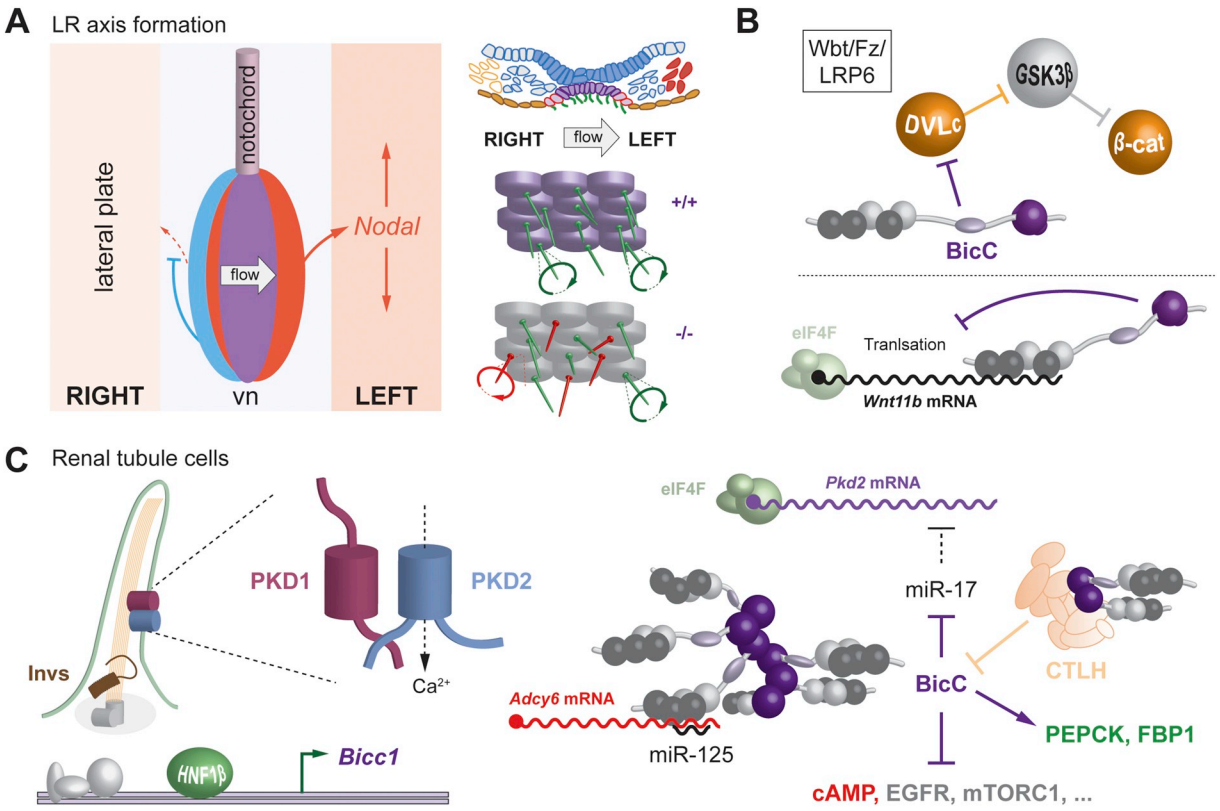

Fig. 2. BicC functions during development and in kidneys.
A. Schematic ventral view (left) and section (right) of node cells. A posterior tilt of motile cilia is essential to generate the leftward fluid flow that inhibits the release of Nodal antagonist (blue) on the right side.
B. BicC polarizes motile cilia, possibly by intercepting cytoplasmic DVL during canonical WNT/β-catenin signaling (top), whereas translational repression of maternal Wnt11b mRNA in *Xenopus* embryos likely influences cell fates during gastrulation (bottom).
C. In renal tubules, HNF1β and PKD1 stimulate *Bicc1* expression. BicC in turn promotes *Pkd2* mRNA translation by inhibiting miR-17, while silencing of *Adcy6* mRNA curbs cAMP synthesis and other growth signals. CTLH complex targets BicC for degradation, possibly to regulate its function in renal gluconeogenesis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

motif in the single-stranded loop of a conserved hairpin [52,53]. However, whether KH1 or KH3 bind other sequences, or a combination of distinct consensus motifs is unknown.

3.2. BicC as a repressor of mRNA translation

On validated target mRNAs, BicC generally reduces translation [15,18,46,47]. *Adcy6* and *PKIα* bind BicC via their proximal 3'UTRs, and repression of luciferase reporters fused to these 3'UTR fragments requires KH domains [15]. Silencing activity also requires the SAM domain, apparently to load BicC-bound mRNA into miRNA-induced silencing complexes (miRISC) with miR-125 and Argonaute [51]. Similarly, if BicC was tethered to an xCR1 3'UTR reporter via MS2 hairpins translational repression relied on C-terminal sequences, including the SAM domain [47]. Moreover, adding an internal ribosomal entry site (IRES) abolished repression, indicating BicC acts on 5′ cap-dependent translation [47], similar to miRNAs [54]. However, given that miRNAs do not efficiently mature in early *Xenopus* embryos, BicC probably represses at least some mRNAs by another mechanism [47]. A yeast-two-hybrid screen for BicC-interacting factors revealed binding to the *Drosophila* homolog of the CNOT3 subunit of CCR4-NOT deadenylase [46]. Several CCR4-NOT subunits and TNRC6A also co-purified with SH-tagged Bicc1 [43]. Complexes of CCR4-NOT with DDX6 can be recruited by the miRISC component TNRC6 or by sequence-specific RNA-binding proteins to repress translation, followed by mRNA decay [54]. Some BicC-bound mRNAs are shorter in wild-type than in *bicc*-deficient egg chambers, but increased mRNA decay has not been reported [46]. BicC also did not shorten the poly(A) tail length or promote the decay of silenced 3'UTR reporters [15,47]. Therefore, the roles of both miRISC and CCR4-NOT in BicC-mediated silencing need further clarification.

3.3. Roles in translational activation

A role for BicC in promoting mRNA translation was discovered in *Xenopus*, where it increases Pkd2 protein levels by directly or indirectly antagonizing miR-17 [16] (Fig. 2E). Expression of the miR-17–92 cluster increases during PKD and is sufficient to induce cysts, whereas its deletion in the *Kif3a* model slows disease [55]. MiR-17 represses factors involved in oxidative phosphorylation and fatty acid oxidation, including PPARα. Treatment of PKD mouse models with miR-17 antagonist or with the PPARα agonist fenofibrate slows cyst formation [56]. However, a role for BicC in regulating PPARα or mitochondrial dysfunction remains to be tested. BicC also binds the translation initiation factor eIF3B and can recruit specific centrosome-associated mRNAs to cap-binding protein eIF4E, the rate-limiting step for translation [57]. Translation initiation factors were also enriched in our proteomic screen for BicC-interacting factors [43]. Future studies should assess whether BicC can switch between translation-activating and -repressive states.

4. BicC localization and stabilization in cytoplasmic granules

4.1. Association with proteins involved in mRNA silencing

BicC localizes in cytoplasmic foci that interact with RNA-processing bodies (PBs) [14,16,51,58]. PBs arise by liquid-liquid phase

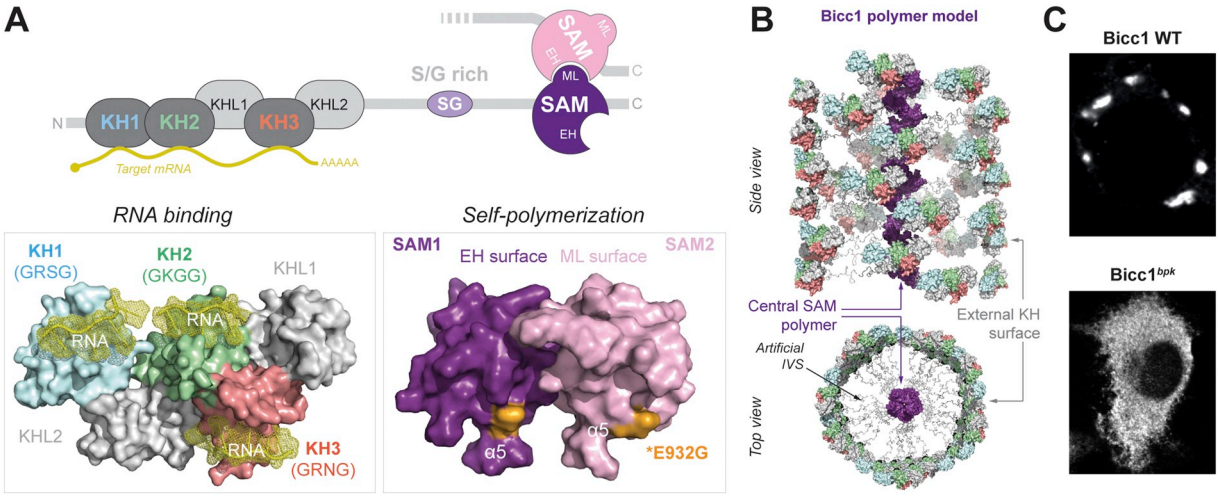

Fig. 3. BicC self-polymerization and its inhibition by the *bpk* mutation.
A. Domain organization (top), and 3D models of the multi-KH and SAM domains of Bicc1 (below) separated by a Serine/Glycine (SG)-rich intervening sequence (IVS). EH and ML surfaces link the SAM domain in head-to-tail polymers. RNA-binding clefts (left) and the mutated SAM domain residue E932 (right) are highlighted.
B. Model of a helical Bicc1 32-mer, with 6 polymerized SAM domains per turn at the center, and peripheral KH domains. The structure of the IVS is unknown.
Distribution of wild-type (top) and *bpk* mutant Bicc1 (bottom) in HEK293T cells.

transitioning of specific scaffold proteins, including DDX6, eIF4E-T, and LSM14A to shield stored mRNAs from ribosomal proteins, translation initiation factors and, depending on context, mRNA decay enzymes [59,60]. In PBs isolated from human HEK293 cells after DDX6 immunostaining, one third of all transcripts are represented, including entire 'regulons' for proteins in common pathways, with sequence-specific RNA-binding proteins acting as zip codes for incorporation or exclusion [60–62]. In *Drosophila* ovary extracts, BicC can coimmunoprecipitate Me31B (DDX6), Cup (eIF4E-T) and Tral (Lsm15, related to human LSM14A) [58]. Consistent with a functional interaction, both *TraI* and *bicc* mutant oocytes mislocalize the EGF-receptor ligand Gurken [58,63]. Ultrastructural analysis confirmed that mRNAs at the core of P-bodies are silenced. By contrast, transcripts of *gurken* remained at the periphery and were translationally activated by Orb specifically in PBs of dorsoanterior cytoplasm [64]. BicC thus likely controls when and where Gurken is translated by regulating Orb [22] and the spatial organization of P-bodies [58]. In mammalian cells, BicC localization at the periphery of PBs could be a mechanism to segregate target mRNAs from a general pool inside.

4.2. BicC self-polymerization

Concentration of BicC in cytoplasmic scaffolds relies on the mid loop (ML) and on the electrostatically complementary end helix (EH) of the SAM domain that mediate head-to-tail self-association in helical polymers [14,51,65] (Fig. 3A, B). Structure modelling of the SAM domain and pull-down assays using recombinant proteins revealed that the aberrant C-terminus of Bicc1^{bpk} blocks self-polymerization, suggesting SAM:SAM interactions are essential in kidneys [51]. Inhibition of polymerization by *bpk* or by engineered mutations in the SAM:SAM interface disperses cytoplasmic Bicc1 foci and accelerates protein turnover (Fig. 3C). Nevertheless, proportionally to its accumulation, *bpk* mutant Bicc1 still interacts with Dvl [51] and suffices for left-right patterning. The conserved E932 residue mutated in a patient with kidney cysts [13] is not part of the SAM:SAM interface but resides nearby so that factors docking at this position would obscure the EH surface (Fig. 3A). Alternatively, EH and/or ML surfaces can be recruited to the CTLH complex for BicC degradation (Fig. 2C), or to ANKS3, suggesting that they are courted by diverse regulatory factors to regulate polymer extension (see below) [43].

5. BicC interactions with other ciliopathy-related proteins

5.1. Heterooligomers of BicC with ANKS3 and ANKS6

ANKS6 (formerly known as SamCystin) was discovered by positional cloning in the PKD/Mhm(cy/+) rat model (formerly Han:SPRD-cy), where arginine 823 is mutated to tryptophane [66]. R823W alters the SAM domain conformation by disrupting contacts between α4 and α5 helices [67]. Two missense mutations also provoke cystic kidneys in mice, including I747N in the SAM domain, and M187K in the fifth Ank repeat. In addition, M187K causes *situs* defects, predominantly right lung isomerism [68,69], suggesting *Anks6* is essential to activate Nodal signaling. ANKS3 emerged from a search for SAM domains that bind ANKS6 [65,67], and its depletion in zebrafish provokes pronephric cysts and heart looping inversions [70,71]. Available reports disagree whether ANKS3 resides primarily in the cytoplasm or also in cilia [72,73]. However, cilia in *anks3*-depleted pronephros lack motility or beat in the wrong direction [70]. In line with a role in cilia polarization, GFP-tagged ANKS3 was enriched at basal bodies of motile cilia in *Xenopus* epidermis [70]. It is important, therefore, to elucidate how ANKS3, ANKS6 and BicC regulate each other.

Coimmunoprecipitation in kidney extracts, structural analysis and yeast-two-hybrid assays established that Bicc1, ANKS3 and ANKS6 interact via both Ank and KH domain arrays and through SAM:SAM interactions [68,74,75]. In the absence of flanking sequences, SAM domains of Bicc1 and ANKS3 form homo- or heterooligomers with comparable affinities. However, in full-length ANKS3, a bulky C-terminus prevents both self-polymerization and the extension of heterooligomers with Bicc1 (Fig. 4A) [75]. In sharp contrast, ANKS6 neither associates with itself, nor with Bicc1 SAM or KH domains. ANKS6 selectively (and with 10-fold higher affinity than Bicc1) binds the SAM domain EH surface of ANKS3 [65,67]. By hijacking the SAM domain of ANKS3, ANKS6 liberates Bicc1 SAM domains for self-polymerization. As ANKS3 holds on to KH domains, it can link ANKS6 to growing Bicc1 homooligomers. ANKS3/ANKS6 heterodimers cannot be extended at their SAM domains. However, independent contacts mediated by Ank repeats interlink such dimers and the associated Bicc1 polymers to each other (Fig. 4B) [75]. Future studies should address whether ANKS3 and ANKS6 dynamically regulate the effect of Bicc1 polymerization on mRNA translation. In turn, specific transcripts may influence associated protein scaffolds, e.g. to regulate mTORC1 or Wnt signaling.

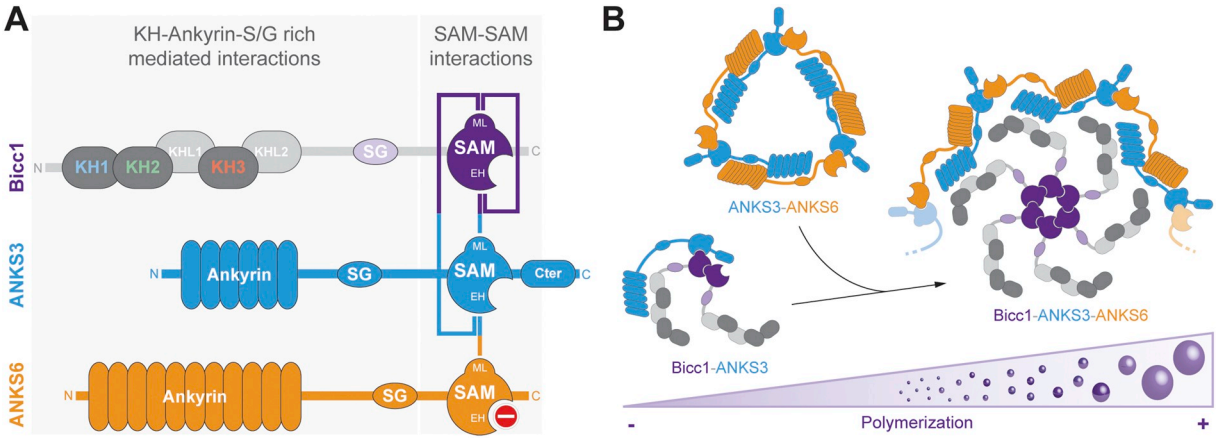

Fig. 4. Rules of engagement of Bicc1 and ANKS6 with ANKS3.
A. Domain organization of ANKS3 and ANKS6, and effects of the C-terminal coiled coil of ANKS3 on possible interactions (bold lines) between SAM domains. Independent contacts of Ankyrin repeats with BicC KH domains or S/G-rich linker regions of their partners are omitted.
B. Cartoon of heterooligomeric complexes of ANKS3 and their re-organization by ANKS6 in higher order complexes of different sizes.

5.2. Interaction of ANKS3/6 with the NIMA-related kinase NEK8

ANKS3 and ANKS6 also bind the Never-In-Mitosis-gene-A (NIMA)-related Ser/Thr kinase Nek8 [70,76]. Nek8 was discovered in juvenile cystic kidney (*jck*) mice carrying the recessive mutation G442 V [77,78]. In proximal tubules, Nek8 accumulates in the cytoplasm, whereas in collecting duct and at the node, it also co-localizes with ciliary INVS and ANKS6 [78,79]. Depending on the context, G442 V may deplete or enrich Nek8 in cilia [79]. However, G442 V is a gain-of-function mutation that neither interferes with Nek8 kinase activity nor with left-right patterning [80]. In sharp contrast, *Nek8* null mutants fail to activate Nodal signaling on either side, leading to right lung isomerism (RLI) despite apparently normal node cilia [78]. This phenotype and its similarity with *Pkd2* mutants and with *Anks6*^{M187K} homozygotes [69] suggest that Pkd2 depends on Nek8/ANKS6 complexes to sense fluid flow. Interestingly, however, an ENU-induced allele encoding the kinase-dead Nek8 I124T mutant, as well as mutations that critically diminish human NEK8 kinase activity provoke cardiac malformations and *left* lung isomerisms (LLI) suggestive of bilateral Nodal signaling [69,81,82]. The finding that loss of *Nek8* or selective inhibition of its kinase activity each elicit opposite left-right phenotypes implies that the left-sided function of ANKS6/NEK8 in flow sensing is counteracted on the right side by NEK8 kinase activity.

So how does NEK8 cooperate with ANKS6? Consistent with a role in flow sensing, Nek8 binds and phosphorylates Pkd2 in kidney extracts, and the G442 V gain-of-function mutation greatly increases Pkd1 and Pkd2 expression in *jck* mice [79]. Conversely, knockdown of *Nek8* in IMCD3 cells blunts store-operated Ca^{2+} influx in the cytosol in response to flow stimulation [78]. Depletion of Pkd2 impairs such cytosolic Ca^{2+} transients to a similar extent [78], and its ion channel activity is essential in kidney and node cilia [83,84] and can be potentiated *ex vivo* by Pkd1 [85]. However, within primary cilia, the permeability of Pkd2 for Ca^{2+} is limited, suggesting that Pkd2 and its ciliary localization regulate Ca^{2+} transients indirectly [86,87].

NEK8 activity is enriched in cilia by a repeat of five Regulator of Chromosome Condensation 1 (RCC1) motifs that directly binds INVS [69,88]. Independently, INVS binds ANKS6 in a complex that is stabilized by Asn hydroxylation of specific Ank repeats by hypoxia-induced factor 1 alpha inhibitor (HIF1AN) [70,76] (Fig. 5A). *Hif1an* knockdown in zebrafish provokes pronephric cysts, suggesting that ANKS6 sequestration in cilia is important [76]. N-terminal Ank repeats of ANKS6 also bind and activate the NEK8 kinase domain, which in turn phosphorylates the NEK8 RCC1 motif as well as ANKS6 to further stabilize ANKS6-INVS interactions [69,80]. Even though kinase-inactive I124T or K33 M mutants may co-localize less efficiently with ANKS6 in cilia, neither ANKS6 nor NEK8 depend on the kinase activity for cilia entry [69]. Instead, studies in mouse kidneys established that INVS and trafficking to cilia increase the accumulation of phosphorylated ANKS6 while drastically reducing the total levels of ANKS6 and its interaction with Bicc1 in the cytosol [73]. Taken together, these observations suggest that phosphorylation by NEK8 is important to clear ANKS6 from the cytoplasm, and that sequestration by INVS in cilia reinforces this inhibition. On the other hand, activation of Pkd2 ion channels by fluid flow may dispatch phospho-ANKS6 from cilia to reorganize BicC polymers and/or promote mRNA silencing in the cytoplasm (Fig. 5B, C). Consistent with a role in regulating the ratio of ciliary and cytoplasmic ANKS6, a rise in intracellular Ca^{2+} can sever INVS from cilia [89]. Furthermore, several disease-associated human NEK8 mutations outside the kinase domain translocate ANKS6 to the cilium base and to cytoplasmic foci [82]. Similarly, R823W mutant mouse ANKS6 which perturbs SAM domain interactions with ANKS3 in cy/+ rats provokes cysts [68], despite the fact that the SAM domain is dispensable for cilia trafficking [69]. Conversely, injection of wild-type Anks6 partially rescues pronephros elongation in *nek8*-depleted *Xenopus* embryos [76]. Given that ANKS6 fails to accumulate in cilia upon NEK8 depletion [82], this points to a protective effect in the cytoplasm.

5.3. Association of Bicc1 and ANKS3 with NEK7

One of the most enriched factors in the Bicc1 protein interactome is NEK7 [43]. NEK7 also associates with ANKS3 [76,90], suggesting ANKS3 and Bicc1 bind NEK7 jointly. NEK7, together with its closest relative NEK6, emerged from a screen for kinases that phosphorylate the translation-activating p70 S6 kinase in response to mitogens [91]. Subsequent functional analysis implicated NEK7 in centriole duplication [92,93], spindle formation and cytokinesis [94], and in suppressing ciliogenesis in proliferating cells during interphase [95]. Consistent with a role in cell division, *Nek7* knockout mouse embryos are severely growth-retarded and rarely develop to term [96]. A large-scale yeast-two-hybrid screen identified 61 individual targets that did not include Bicc1 or ANKS3 [97], as expected if NEK7 binds their complex. Binding to NEK7 promotes a phosphatase-insensitive post-translational modification of ANKS3, while ANKS3 in turn enriches NEK7 in the cytoplasm by preventing nuclear accumulation [90]. A direct link of NEK7 to ciliopathies is elusive, but in *C. elegans*, anks3 with nek7, and anks6 together with nek8 and invs regulate actin organization by inhibiting the Rho-related GTPase Cdc42 [98]. RhoA and Cdc42 promote cysts [8,99] and are hyperactive in ADPKD [100]. It will be important, therefore, to investigate how they escape from inhibition by ANKS3/ANKS6.

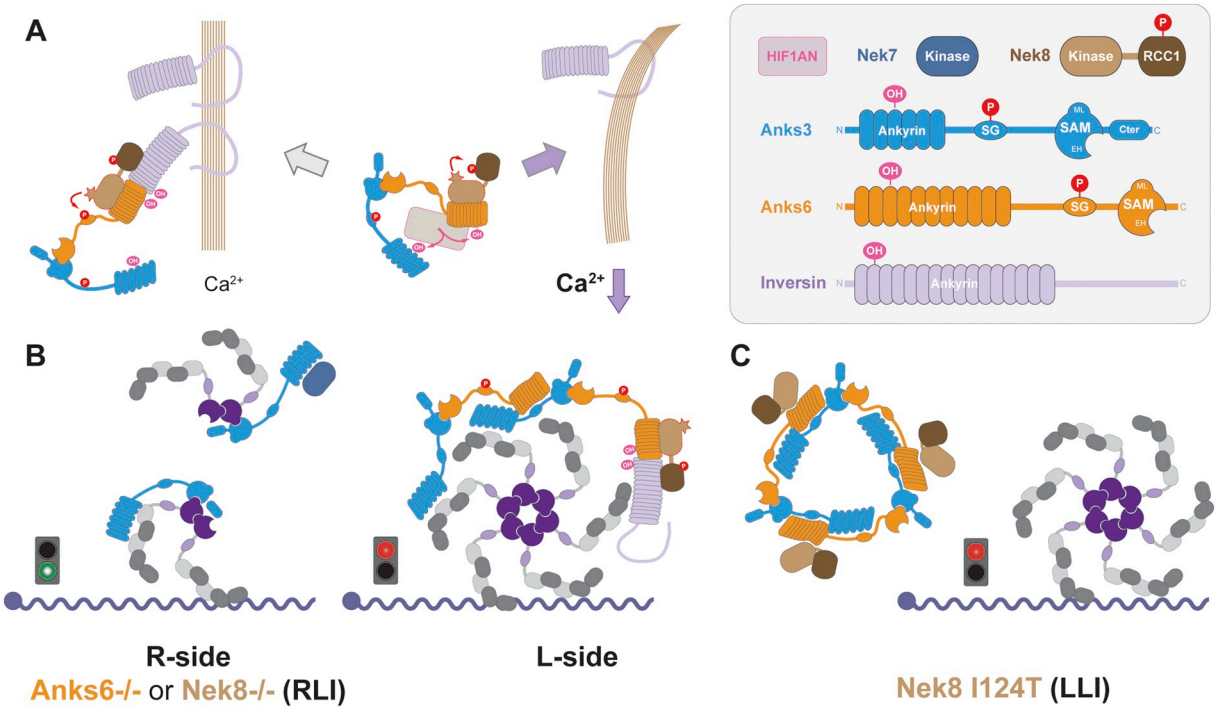

Fig. 5. ANKS6 localization and its predicted influence on BicC heterooligomers.
ANKS6 and NEK8 localize to cilia (brown stripes) by associating with INVS. ANKS6 activates NEK8 kinase which in turn phosphorylates itself and a Ser- and Gly-rich region (SG) of ANKS6. Hydroxylation of specific Ank domains of ANKS6 and INVS by HIF1AN further increases their association. HIF1AN also modifies ANKS3. Stimulation of cilia may facilitate recycling of phospho-ANKS6 to the cytoplasm (with or without INVS and ANKS3) in a Pkd2/Ca^{2+}-dependent manner.
In the absence of ANKS6, both the Ank and SAM domains of cytoplasmic ANKS3 engage BicC. The absence of fluid flow, or loss of ANKS6 or NEK8 thus may irreversibly inhibit BicC polymerization. By contrast, when ANKS6 accumulates in the cytoplasm, the higher affinity of its SAM domain titrates ANKS3, thereby liberating the BicC SAM domain to self-polymerize and potentiate silencing (red light) of target mRNAs (wavy line). Phosphorylation by NEK8 may shape the architecture of BicC heterooligomers by hindering the SG region of ANKS6 to bind Ank domains of ANKS3 or INVS.
The accumulation of kinase-dead NEK8/ANKS6 complexes in *Nek8*^{I124T} mutant mice provokes left lung isomerism (LLI), possibly by trapping ANKS3 in non-functional aggregates that allow Bicc1 to self-polymerize independently of fluid flow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

6. Conclusion and perspectives

Genetic studies of BicC, ANKS3 and ANKS6 loss-of-function phenotypes in different model organisms in kidneys and during left-right development laid the foundation to investigate how these and other CKD-relevant interacting factors regulate each other. Among ciliopathy proteins, BicC stands out as the only factor known to directly mediate post-transcriptional gene regulation at the level of mRNA translation, and as one of the few that are not 'simply' acting as protein scaffolds or as integral components of cilia or centrosome formation. ANKS3 and ANKS6 emerged as new candidate regulators of BicC higher order complexes, notably to control liquid-liquid phase transitions, although functional readouts to directly test this are still limited. We also highlighted interacting kinases of the NEK family, because their PKD-relevant substrates are unknown, and because BicC/ANKS3 and ANKS3/ANKS6 complexes emerged as likely suspects. Equally important will be to assess how interactions among these SAM domain proteins regulate NEK8 and NEK7 activities and their roles in coupling centrosome formation and ciliogenesis to cell cycle progression. Another challenge is to map how known interactions with INVS fit into the picture, whether the ciliary INVS compartment is the final destination or a storage site, or a transit station for ANKS6 complexes (Fig. 5), and whether it couples cilia and Ca^{2+} to the regulation of BicC activity in mRNA silencing.

Finally, it will be important to elucidate how these SAM domain proteins control metabolism. Since mRNA translation and cell metabolism are coupled to coordinate tissue growth, BicC unlikely regulates only one without the other. Unbiased screens for BicC targets and metabolomic profiling should identify the steps where BicC is rate-limiting to control metabolic flux, and how they integrate ciliary inputs. Metabolic re-programming is a cancer hallmark and accelerates PKD progression, but metabolic differences among ciliopathies are understudied. The Lewis polycystic kidney rat model of NPHP9 shows decreased arginine and proline and linoleic acid metabolism and accelerated urea cycle, while few TCA intermediates are down- or upregulated [101]. In *Anks3*-depleted IMCD3 cells, amino acid levels generally increase, whereas branched chain amino acids activating mTORC1 are diminished, correlating with the fact that cell proliferation and kidney sizes decrease in NPHP, rather than being increased. Apart from pyruvate and fructose, glycolytic and TCA metabolites were unaltered, and ECAR measurements revealed no increase in glycolysis [102]. However, increased N6-methyladenosine levels point to altered DNA and RNA metabolism [102]. Similar alterations in amino acid and purine metabolism were observed in *Anks6*-depleted cells, pointing to a new role for ANKS3 and ANKS6 in inhibiting DNA damage [103]. Genetic stability also depends on PKD1 and PKD2 [104,105]. Mapping multivalent interactions in the BicC-ANKS3-ANKS6 network and how they control these and possibly other cancer hallmarks will likely reveal new opportunities for targeted interventions.

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References

[1] T. Seeger-Nukpezah, D.M. Geynisman, A.S. Nikonova, T. Benzing, E.A. Golemis, The hallmarks of cancer: relevance to the pathogenesis of polycystic kidney disease, Nat Rev Nephrol 11 (2015) 515–534.

[2] T.-M. Yu, et al., Risk of cancer in patients with polycystic kidney disease: a propensity-score matched analysis of a nationwide, population-based cohort study, Lancet Oncol 17 (2016) 1419–1425.

[3] D.A. Braun, F. Hildebrandt, Ciliopathies, Cold Spring Harb Perspect Biol 9 (2017).

[4] C. Willey, S. Kamat, R. Stellhorn, J. Blais, Analysis of Nationwide data to determine the incidence and diagnosed prevalence of autosomal dominant polycystic kidney disease in the USA: 2013–2015, Kidney Dis 5 (2019) 107–117.

[5] R. Pala, N. Alomari, S.M. Nauli, Primary cilium-dependent signaling mechanisms, Int J Mol Sci 18 (2017).

[6] R. Mangoo-Karim, M. Uchic, C. Lechene, J.J. Grantham, Renal epithelial cyst formation and enlargement in vitro: dependence on cAMP, Proc Natl Acad Sci U S A 86 (1989) 6007–6011.

[7] F.T. Chebib, C.R. Sussman, X. Wang, P.C. Harris, V.E. Torres, Vasopressin and disruption of calcium signalling in polycystic kidney disease, Nat Rev Nephrol 11 (2015) 451–464.

[8] J. Cai, et al., A RhoA–YAP–c-Myc signaling axis promotes the development of polycystic kidney disease, Genes Dev 32 (2018) 781–793.

[9] K. Shinohara, H. Hamada, Cilia in left–right symmetry breaking, Cold Spring Harb Perspect Biol 9 (2017).

[10] J. Nauta, Y. Ozawa, W.E. Sweeney, J.C. Rutledge, E.D. Avner, Renal and biliary abnormalities in a new murine model of autosomal recessive polycystic kidney disease, Pediatr Nephrol 7 (1993) 163–172.

[11] L. Flaherty, E.C. Bryda, D. Collins, U. Rudofsky, J.C. Montogomery, New mouse model for polycystic kidney disease with both recessive and dominant gene effects, Kidney Int 47 (1995) 552–558.

[12] C. Cogswell, et al., Positional cloning of jcpk / bpk locus of the mouse, Mamm Genome 14 (2003) 242–249.

[13] M.R.-C. Kraus, et al., Two mutations in human BICC1 resulting in Wnt pathway hyperactivity associated with cystic renal dysplasia, Hum Mutat 33 (2012) 86–90.

[14] C. Maisonneuve, et al., Bicaudal C, a novel regulator of Dvl signaling abutting RNA-processing bodies, controls cilia orientation and leftward flow, Development 136 (2009) 3019–3030.

[15] N. Piazzon, C. Maisonneuve, I. Guilleret, S. Rotman, D.B. Constam, Bicc1 links the regulation of cAMP signaling in polycystic kidneys to microRNA-induced gene silencing, J Mol Cell Biol 4 (2012) 398–408.

[16] U. Tran, et al., The RNA-binding protein bicaudal C regulates polycystin 2 in the kidney by antagonizing miR-17 activity, Development 137 (2010) 1107–1116.

[17] D.J. Bouvrette, V. Sittaramane, J.R. Heidel, A. Chandrasekhar, E.C. Bryda, Knockdown of bicaudal C in zebrafish (Danio rerio) causes cystic kidneys: a nonmammalian model of polycystic kidney disease, Comp Med 60 (2010) 96–106.

[18] C. Gamberi, D.R. Hipfner, M. Trudel, W.D. Lubell, Bicaudal C mutation causes myc and TOR pathway up-regulation and polycystic kidney disease-like phenotypes in Drosophila, PLoS Genet 13 (2017) e1006694.

[19] J. Mohler, E.F. Wieschaus, Dominant maternal-effect mutations of Drosophila melanogaster causing the production of double-abdomen embryos, Genetics 112 (1986) 803–822.

[20] M. Mahone, E.E. Saffman, P.F. Lasko, Localized Bicaudal-C RNA encodes a protein containing a KH domain, the RNA binding motif of FMR1, EMBO J 14 (1995) 2043–2055.

[21] E.E. Saffman, et al., Premature translation of oskar in oocytes lacking the RNA-binding protein bicaudal-C, Mol Cell Biol 18 (1998) 4855–4862.

[22] S. Castagnetti, A. Ephrussi, Orb and a long poly(a) tail are required for efficient *oskar* translation at the posterior pole of the *Drosophila* oocyte, Development 130 (2003) 835–843.

[23] S.M. McDermott, C. Meignin, J. Rappsilber, I. Davis, Drosophila Syncrip binds the gurken mRNA localisation signal and regulates localised transcripts during axis specification, Biol Open 1 (2012) 488–497.

[24] O. Wessely, E.M. De Robertis, The Xenopus homologue of Bicaudal-C is a localized maternal mRNA that can induce endoderm formation, Development 127 (2000) 2053–2062.

[25] S. Yaguchi, J. Yaguchi, K. Inaba, Bicaudal-C is required for the formation of anterior neurogenic ectoderm in the sea urchin embryo, Sci Rep 4 (2015) 6852.

[26] S. Lienkamp, A. Ganner, G. Inversin Walz, Wnt signaling and primary cilia, Differentiation 83 (2012) S49–S55.

[27] S. Park, S. Blaser, M.A. Marchal, D.W. Houston, M.D. Sheets, A gradient of maternal Bicaudal-C controls vertebrate embryogenesis via translational repression of mRNAs encoding cell fate regulators, Development 143 (2016) 864–871.

[28] N. Sugiyama, T. Tsukiyama, T.P. Yamaguchi, T. Yokoyama, The canonical Wnt signaling pathway is not involved in renal cyst development in the kidneys of inv mutant mice, Kidney Int 79 (2011) 957–965.

[29] A. Li, et al., Canonical Wnt inhibitors ameliorate cystogenesis in a mouse ortholog of human ADPKD, JCI Insight 3 (2018).

[30] S. Kim, et al., The polycystin complex mediates Wnt/Ca2+ signalling, Nat Cell Biol 18 (2016) 752–764.

[31] P. Lian, et al., Loss of polycystin-1 inhibits Bicc1 expression during mouse development, PLoS One 9 (2014) e88816–e88816.

[32] F. Verdeguer, et al., A mitotic transcriptional switch in polycystic kidney disease, Nat Med 16 (2010) 106–110.

[33] W.G. Richards, et al., Epidermal growth factor receptor activity mediates renal cyst formation in polycystic kidney disease, J Clin Invest 101 (1998) 935–939.

[34] W.E. Sweeney, et al., Phenotypic analysis of conditionally immortalized cells isolated from the BPK model of ARPKD, Am J Phys Cell Phys 281 (2001) C1695–C1705.

[35] S. Ryan, S. Verghese, N.L. Cianciola, C.U. Cotton, C.R. Carlin, Autosomal recessive polycystic kidney disease epithelial cell model reveals multiple basolateral epidermal growth factor receptor sorting pathways, Mol Biol Cell 21 (2010) 2732–2745.

[36] L.A. Lemaire, et al., Bicaudal C1 promotes pancreatic NEUROG3+ endocrine progenitor differentiation and ductal morphogenesis, Development 142 (5) (2016) 858–870, https://doi.org/10.1242/dev.114611.

[37] I. Rowe, et al., Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy, Nat Med 19 (2013) 488–493.

[38] C. Podrini, et al., Dissection of metabolic reprogramming in polycystic kidney disease reveals coordinated rewiring of bioenergetic pathways, Commun Biol 1 (2018) 194.

[39] L.F. Menezes, C.-C. Lin, F. Zhou, G.G. Germino, Fatty acid oxidation is impaired in an orthologous mouse model of autosomal dominant polycystic kidney disease, EBioMedicine 5 (2016) 183–192.

[40] L.F. Menezes, et al., Network analysis of a Pkd1-mouse model of autosomal dominant polycystic kidney disease identifies HNF4α as a disease modifier, PLoS Genet 8 (2012) e1003053–e1003053.

[41] Y. Ishimoto, et al., Mitochondrial abnormality facilitates cyst formation in autosomal dominant polycystic kidney disease, Mol Cell Biol 37 (2017).

[42] A. Hofherr, et al., The mitochondrial transporter SLC25A25 links ciliary TRPP2 signaling and cellular metabolism, PLoS Biol 16 (2018) e2005651.

[43] L.C. Leal-Esteban, B. Rothé, S. Fortier, M. Isenschmid, D.B. Constam, Role of Bicaudal C1 in renal gluconeogenesis and its novel interaction with the CTLH complex, PLoS Genet 14 (2018) e1007487.

[44] O. Santt, et al., The yeast GID complex, a novel ubiquitin ligase (E3) involved in the regulation of carbohydrate metabolism, Mol Biol Cell 19 (2008) 3323–3333.

[45] S.-J. Chen, X. Wu, B. Wadas, J.-H. Oh, A. Varshavsky, An N-end rule pathway that recognizes proline and destroys gluconeogenic enzymes, Science 355 (2017) eaal3655.

[46] J. Chicoine, et al., Bicaudal-C recruits CCR4-NOT Deadenylase to target mRNAs and regulates oogenesis, cytoskeletal organization, and its own expression, Dev Cell 13 (2007) 691–704.

[47] Y. Zhang, et al., Bicaudal-C spatially controls translation of vertebrate maternal mRNAs, RNA 19 (2013) 1575–1582.

[48] W. Cheng, G. Chen, H. Jia, X. He, Z. Jing, DDX5 RNA helicases: Emerging roles in viral infection, Int J Mol Sci 19 (2018).

[49] G. Nicastro, I.A. Taylor, A. Ramos, KH-RNA interactions: Back in the groove, Curr Opin Struct Biol 30 (2015) 63–70.

[50] K. Nakel, S.A. Hartung, F. Bonneau, C.R. Eckmann, E. Conti, Four KH domains of the C. elegans Bicaudal-C ortholog GLD-3 form a globular structural platform, RNA 16 (2010) 2058–2067.

[51] B. Rothé, et al., Bicc1 polymerization regulates the localization and silencing of bound mRNA, Mol Cell Biol 35 (2015) 3339–3353.

[52] Y. Zhang, S. Park, S. Blaser, M.D. Sheets, Determinants of RNA binding and translational repression by the Bicaudal-C regulatory protein, J Biol Chem 289 (2014) 7497–7504.

[53] M.E. Dowdle, et al., A single KH domain in Bicaudal-C links mRNA binding and translational repression functions to maternal development, Development 146 (2019) dev172486.

[54] S. Jonas, E. Izaurralde, Towards a molecular understanding of microRNA-mediated gene silencing, Nat Rev Genet 16 (2015) 421–433.

[55] V. Patel, et al., miR-17 92 miRNA cluster promotes kidney cyst growth in polycystic kidney disease, Proc Natl Acad Sci 110 (2013) 10765–10770.

[56] S. Hajarnis, et al., MicroRNA-17 family promotes polycystic kidney disease progression through modulation of mitochondrial metabolism, Nat Commun 8 (2017) 1–15.

[57] D. Iaconis, et al., The centrosomal OFD1 protein interacts with the translation machinery and regulates the synthesis of specific targets, Sci Rep 7 (2017) 1224.

[58] J.-M. Kugler, J. Chicoine, P. Lasko, Bicaudal-C associates with a trailer hitch/Me31B complex and is required for efficient Gurken secretion, Dev Biol 328 (2009) 160–172.

[59] N. Standart, D. Weil, P-bodies: cytosolic droplets for coordinated mRNA storage, Trends Genet 34 (2018) 612–626.

[60] C. Wang, et al., Context-dependent deposition and regulation of mRNAs in P-bodies, Elife 7 (2018).

[61] A. Hubstenberger, et al., P-Body Purification Reveals the Condensation of Repressed mRNA Regulons, Mol Cell 68 (2017) 144–157 e5.

[62] A. Hubstenberger, et al., P-Body Purification Reveals the Condensation of Repressed mRNA Regulons, Mol Cell 68 (2017) 144–157 e5.

[63] J.E. Wilhelm, M. Buszczak, S. Sayles, Efficient protein trafficking requires trailer hitch, a component of a ribonucleoprotein complex localized to the ER in Drosophila, Dev Cell 9 (2005) 675–685.

[64] T.T. Weil, et al., Drosophila patterning is established by differential association of mRNAs with P bodies, Nat Cell Biol 14 (2012) 1305–1313.

[65] M.J. Knight, C. Leettola, M. Gingery, H. Li, J.U. Bowie, A human sterile alpha motif domain polymerizome, Protein Sci 20 (2011) 1697–1706.

[66] J.H. Brown, et al., Missense mutation in sterile α motif of novel protein SamCystin is associated with polycystic kidney disease in (cy/+) rat, J Am Soc Nephrol 16 (2005) 3517–3526.

[67] C.N. Leettola, M. Knight, D. Cascio, S. Hoffman, J.U. Bowie, Characterization of the SAM domain of the PKD-related protein ANKS6 and its interaction with ANKS3, BMC Struct Biol 14 (2014) 17.

[68] Z. Bakey, et al., The SAM domain of ANKS6 has different interacting partners and mutations can induce different cystic phenotypes, Kidney Int 88 (2015) 299–310.
[69] P.G. Czarnecki, et al., ANKS6 is the critical activator of NEK8 kinase in embryonic situs determination and organ patterning, Nat Commun 6 (2015) 6023.
[70] T.A. Yakulov, et al., Anks3 interacts with nephronophthisis proteins and is required for normal renal development, Kidney Int 87 (2015) 1191–1200.
[71] H.E. Shamseldin, T.A. Yakulov, A. Hashem, G. Walz, F.S. Alkuraya, ANKS3 is mutated in a family with autosomal recessive laterality defect, Hum Genet 135 (2016) 1233–1239.
[72] L. Delestré, et al., ANKS3 co-Localises with ANKS6 in mouse renal cilia and is associated with vasopressin signaling and apoptosis in vivo in mice, PLoS One 10 (2015) e0136781.
[73] Y. Nakajima, H. Kiyonari, Y. Mukumoto, T. Yokoyama, The Inv compartment of renal cilia is an intraciliary signal activating center to phosphorylate ANKS6, Kidney Int (2018), https://doi.org/10.1016/j.kint.2017.11.016.
[74] E.E. Stagner, D.J. Bouvrette, J. Cheng, E.C. Bryda, The polycystic kidney disease-related proteins Bicc1 and SamCystin interact, Biochem Biophys Res Commun 383 (2009) 16–21.
[75] B. Rothé, et al., Crystal structure of Bicc1 SAM polymer and mapping of interactions between the ciliopathy-associated proteins Bicc1, ANKS3, and ANKS6, Structure 26 (2018) 209–224.e6.
[76] S. Hoff, et al., ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and NPHP3. *Nat*, Genet 45 (2013) 951–956.
[77] S. Liu, et al., A defect in a novel Nek-family kinase causes cystic kidney disease in the mouse and in zebrafish, Development 129 (2002) 5839–5846.
[78] D.K. Manning, et al., Loss of the ciliary kinase Nek8 causes left-right asymmetry defects, J Am Soc Nephrol 24 (2013) 100–112.
[79] E. Sohara, et al., Nek8 regulates the expression and localization of polycystin-1 and polycystin-2, J Am Soc Nephrol 19 (2008) 469–476.
[80] D. Zalli, R. Bayliss, A.M. Fry, The Nek8 protein kinase, mutated in the human cystic kidney disease nephronophthisis, is both activated and degraded during ciliogenesis, Hum Mol Genet 21 (2012) 1155–1171.
[81] V. Frank, et al., Mutations in NEK8 link multiple organ dysplasia with altered hippo signalling and increased c-MYC expression, Hum Mol Genet 22 (2013) 2177–2185.
[82] V. Grampa, et al., Novel NEK8 mutations cause severe syndromic renal cystic dysplasia through YAP dysregulation, PLoS Genet 12 (2016) e1005894.
[83] S. Yoshiba, et al., Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2, Science 338 (80) (2012) 226–231.
[84] R.V. Walker, et al., Ciliary exclusion of Polycystin-2 promotes kidney cystogenesis in an autosomal dominant polycystic kidney disease model. *Nat*, Commun 10 (2019) 4072.
[85] Z. Wang, et al., The ion channel function of polycystin-1 in the polycystin-1/polycystin-2 complex, EMBO Rep (2019) 20.
[86] M. Delling, et al., Primary cilia are not calcium-responsive mechanosensors, Nature 531 (2016) 656–660.
[87] X. Liu, et al., Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium, Elife 7 (2018).
[88] D. Shiba, D.K. Manning, H. Koga, D.R. Beier, T. Yokoyama, Inv acts as a molecular anchor for Nphp3 and Nek8 in the proximal segment of primary cilia, Cytoskeleton 67 (2010) NA-NA.
[89] D. Shiba, et al., Localization of Inv in a distinctive intraciliary compartment requires the C-terminal ninein-homolog-containing region, J Cell Sci 122 (2009) 44–54.
[90] H. Ramachandran, et al., Anks3 alters the sub-cellular localization of the Nek7 kinase, Biochem Biophys Res Commun 464 (2015) 901–907.
[91] C. Belham, M.J. Comb, J. Avruch, Identification of the NIMA family kinases NEK6/7 as regulators of the p70 ribosomal S6 kinase, Curr Biol 11 (2001) 1155–1167.
[92] J. Loncarek, P. Hergert, V. Magidson, A. Khodjakov, Control of daughter centriole formation by the pericentriolar material, Nat Cell Biol 10 (2008) 322–328.
[93] S. Kim, S. Kim, S. Kim, K. Rhee, NEK7 is essential for centriole duplication and centrosomal accumulation of pericentriolar material proteins in interphase cells, J Cell Sci 124 (2011) 3760–3770.
[94] L. O'Regan, A.M. Fry, The Nek6 and Nek7 protein kinases are required for robust mitotic spindle formation and cytokinesis, Mol Cell Biol 29 (2009) 3975–3990.
[95] A. Gupta, Y. Tsuchiya, M. Ohta, G. Shiratsuchi, D. Kitagawa, NEK7 is required for G1 progression and procentriole formation, Mol Biol Cell 28 (2017) 2123–2134.
[96] H. Salem, et al., Nek7 kinase targeting leads to early mortality, cytokinesis disturbance and polyploidy, Oncogene 29 (2010) 4046–4057.
[97] E.E. De Souza, et al., Characterization of the human NEK7 interactome suggests catalytic and regulatory properties distinct from those of NEK6, J Proteome Res 13 (2014) 4074–4090.
[98] V. Lažetić, B.B. Joseph, S.M. Bernazzani, D.S. Fay, Actin organization and endocytic trafficking are controlled by a network linking NIMA-related kinases to the CDC-42-SID-3/ACK1 pathway, PLoS Genet 14 (2018) e1007313.
[99] K.K. Rogers, T.-S. Jou, W. Guo, J.H. Lipschutz, The rho family of small GTPases is involved in epithelial cystogenesis and tubulogenesis, Kidney Int 63 (2003) 1632–1644.
[100] A. Luyten, et al., Aberrant regulation of planar cell polarity in polycystic kidney disease, J Am Soc Nephrol 21 (2010) 1521–1532.
[101] H. Abbiss, et al., Untargeted gas chromatography–mass spectrometry-based metabolomics analysis of kidney and liver tissue from the Lewis polycystic kidney rat, J Chromatogr B Anal Technol Biomed Life Sci (2019) 25–32 1118–1119.
[102] M. Schlimpert, et al., Metabolic phenotyping of Anks3 depletion in mIMCD-3 cells - a putative Nephronophthisis candidate, Sci Rep 8 (2018).
[103] M. Schlimpert, et al., Metabolic perturbations caused by depletion of nephronophthisis factor Anks6 in mIMCD3 cells, Metabolomics 15 (2019).
[104] L. Battini, et al., Loss of polycystin-1 causes centrosome amplification and genomic instability, Hum Mol Genet 17 (2008) 2819–2833.
[105] S. Burtey, et al., Centrosome overduplication and mitotic instability in PKD2 transgenic lines, Cell Biol Int 32 (2008) 1193–1198.
[106] L.M. Guay-Woodford, C.J. Wright, G. Walz, G.A. Churchill, Quantitative trait loci modulate renal cystic disease severity in the mouse bpk model, J Am Soc Nephrol 11 (2000) 1253–1260.
[107] S. Neudecker, et al., Transgenic overexpression of Anks6(p.R823W) causes polycystic kidney disease in rats, Am J Pathol 177 (2010) 3000–3009.
[108] E.Z. Taskiran, et al., Mutations in *ANKS6* cause a Nephronophthisis-like phenotype with ESRD, J Am Soc Nephrol 25 (2014) 1653–1661.
[109] I. Ivanovska, et al., MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression, Mol Cell Biol 28 (2008) 2167–2174.
[110] E.A. Otto, et al., NEK8 mutations affect ciliary and centrosomal localization and may cause nephronophthisis, J Am Soc Nephrol 19 (2008) 587–592.
[111] S. Habbig, et al., The ciliopathy disease protein NPHP9 promotes nuclear delivery and activation of the oncogenic transcriptional regulator TAZ, Hum Mol Genet 21 (2012) 5528–5538.
[112] H.J.C. Choi, et al., NEK8 links the ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies, Mol Cell 51 (2013) 423–439.