Published in final edited form as:
Cytoskeleton (Hoboken). 2016 January ; 73(1): 3–22. doi:10.1002/cm.21271.

Mammalian Axoneme Central Pair Complex Proteins: Broader Roles Revealed by Gene Knockout Phenotypes

Maria E. Teves[1], **David R. Nagarkatti-Gude**[1,2], **Zhibing Zhang**[1,2], and **Jerome F. Strauss III**[1,2,*]

[1]Department of Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, Virginia

[2]Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, Virginia

Abstract

The axoneme genes, their encoded proteins, their functions and the structures they form are largely conserved across species. Much of our knowledge of the function and structure of axoneme proteins in cilia and flagella is derived from studies on model organisms like the green algae, *Chlamydomonas reinhardtii*. The core structure of cilia and flagella is the axoneme, which in most motile cilia and flagella contains a 9 + 2 configuration of microtubules. The two central microtubules are the scaffold of the central pair complex (CPC). Mutations that disrupt CPC genes in *Chlamydomonas* and other model organisms result in defects in assembly, stability and function of the axoneme, leading to flagellar motility defects. However, targeted mutations generated in mice in the orthologous CPC genes have revealed significant differences in phenotypes of mutants compared to *Chlamydomonas*. Here we review observations that support the concept of cell-type specific roles for the CPC genes in mice, and an expanded repertoire of functions for the products of these genes in cilia, including non-motile cilia, and other microtubule-associated cellular functions.

Keywords

cilia; flagella; axoneme; central pair complex

Introduction

Cilia are specialized hair-like structures, classified as either motile or immotile. Motile cilia have a 9 + 2 organization of microtubules and are present on the respiratory epithelium, ependymal cells, in the female reproductive tract, and in flagellated cells [Brown and Witman, 2014]. Nodal cilia with a 9 + 0 axoneme structure are also a type of motile cilia. Immotile cilia have a 9 + 0 axoneme structure and include primary cilia present on most vertebrate cells [Satir and Christensen, 2007; Brown and Witman, 2014] (Fig. 1).

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[*]Address correspondence to: J.F. Strauss III, Department of Obstetrics and Gynecology, Virginia Commonwealth University, Richmond., Virginia jerome.strauss@vcuhealth.org.

The structure of cilia and flagella has been highly conserved across evolution. The homology between flagellar proteins of unicellular organisms, and mammalian ciliary and flagellar proteins suggests that the main functional role of the genes encoding the axoneme proteins have been preserved [Ibanez-Tallon et al., 2003; Mitchell, 2004; Carvalho-Santos et al., 2011]. Immotile cilia and motile cilia/flagella from organisms in most taxonomic groups share a similar basic structure comprised of an arrangement of nine doublets of microtubules. Motile cilia/flagella have, in addition, associated projections from the 9 doublets including dynein arms, radial spokes, and nexin links surrounding a pair of singlet microtubule known as the central pair complex (CPC) [Lee, 2011] (Fig.1). Most of the genetic, structural, and biochemical work on the central pair has taken advantage of the model organism, the green algae, *Chlamydomonas reinhardtii* [Mitchell and Smith, 2009].

The two microtubules of the central pair are structurally and biochemically dimorphic, and by convention are given separate designations (i.e., C1 and C2) [Dutcher et al., 1984; Goodenough and Heuser, 1985; Dutcher, 1995; Smith and Lefebvre, 1997a,b]. Several CPC projections, in addition to a sheath partly surrounding the CPC, were identified using classical electron microscopy and 2D averages of CPC cross-sections [Mitchell and Smith, 2009]. Recently, new CPC projections have been identified by cryoelectron tomography and 3D average imaging [Carbajal-González et al., 2013]. This important study also revealed that the sheath is not an independent density connecting adjacent projections, but rather is the outermost part of projections extending from the C1 and C2 microtubules.

In summary (Fig. 2), the most recently described CPC structure shows that the C1 microtubule has two longer projections (1a and 1b) and four shorter projections (1c, 1d, 1e and 1f). Similar to the C1 microtubule, the C2 microtubule possesses two larger projection (2a and 2b), and three shorter projections (2c, 2d and 2e). There is a bridge linking the C1 and C2 microtubules [Carbajal-González et al., 2013].

The motility and waveforms produced by the beating cilia/flagellum are believed to be the result of coordinated regulation of the dynein arms [Huang et al., 1979; Curry and Rosenbaum, 1993; Porter, 1996; Habermacher and Sale, 1997; Smith and Lefebvre, 1997a,b; Porter and Sale, 2000; Mitchell, 2004]. It has been proposed that the central apparatus and the radial spokes function as a distributor to provide local signals that regulate the activity of specific dynein arms [Smith, 2002; Mitchell, 2004; Lesich et al., 2010]. In this context, mutations in genes encoding CPC proteins (described in detail below) have provided valuable evidence about the important roles that these proteins play in ciliary/flagellar motility.

Ciliopathies encompas a range of human diseases associated with mutations [Lee, 2011; Waters and Beales, 2011; Raidt et al., 2014]. They are characterized by abnormal formation and function of cilia [Lee, 2011]. Because cilia perform diverse biological roles, affected patients have several and variable phenotypes. Primary cilia, serve principally as key coordinators of signaling pathways during development and are also important in tissue homeostasis [Gerdes et al., 2009; Satir and Christensen, 2007; Satir et al., 2010; Oh and Katsanis, 2012]. Thus, defects in primary cilia can lead to a broader set of developmental and adult phenotypes that include polycystic liver and kidney disease, retinal degeneration, skeletal dysplasia and obesity. (Fig. 1). On the other hand, the function of motile cilia and flagella is principally related to movement of fluids over specialized epithelia and swimming of single cells [Silflow and Lefebvre, 2001; Satir and Christensen, 2007; Vincensini et al., 2011]. Motile cilia dysfunction results in bronchiectasis, and patients suffer from rhinitis, sinusitis and otitis media. Other complications include hydrocephalus, *situs inversus* and infertility [Badano et al., 2006; Brown and Witman, 2014] (Fig. 1). The literature on ciliary dysfunction in animal models and human studies grew significantly during the last decade [Brown and Witman, 2014]. In this review, we have compiled recent information that supports the concepts of cell-type specific roles of the CPC, and an expanded repertoire of functions for genes encoding CPC proteins in mammals.

Ciliary and Flagellar Beat

Ciliary/flagellar beating originates from the sliding of the doublet microtubules driven by the dynein arms in a coordinated fashion [Lee, 2011]. While the molecules involved in the regulatory mechanisms are similar in the mammalian cilia/flagella and *Chlamydomonas* flagella, there are some important differences [Salathe, 2007; Lee, 2011]. One major difference is that the CPC rotates in *Chlamydomonas*, but is fixed in position in mammals, which requires a different mechanism to account for asymmetrical activation of dynein arms driving microtubule sliding [Inaba, 2011; Lee, 2011]. The 12 μm *Chlamydomonas* flagella can beat with two different waveforms at frequencies near to 60 Hz, enabling propulsion in opposite directions [Silflow and Lefebvre, 2001]. Human respiratory cilia from intact epithelia are approximately 7 μm long and beat with a single waveform at much lower frequencies (~10–15 Hz) to transport mucus out of the airways [Wanner et al., 1996; Salathe, 2007]. Motility regulation through the CPC depends on pH, cAMP and calcium signaling for both cilia and flagella [Lee, 2011]. Increases in concentration of cAMP activate protein kinase A (PKA), which then phosphorylates specific target proteins in the axoneme [Salathe, 2007]. In human cilia, [Wanner et al., 1996; Salathe, 2007] and sperm [Turner, 2006], an increase in cAMP stimulates beat frequency, while causing a decrease in *Chlamydomonas* [Wanner et al., 1996; Lee, 2011]. Similarly, influx of Ca^{2+} stimulates motility of human cilia [Wanner et al., 1996; Lee, 2011; Inaba, 2015] while causing *Chlamydomonas* flagella to change from an asymmetric waveform to a symmetric waveform [Wanner et al., 1996; Silflow and Lefebvre, 2001; Lee, 2011; Inaba, 2015]. In sperm, Ca^{2+} influx promotes flagellar motility [Turner, 2006; Inaba, 2015]. However, flagella are quiescent at high calcium concentrations [Nakano et al., 2003; Turner, 2006; Inaba, 2015]. The effects of calcium on the axoneme are known to be mediated by the interaction with calmodulin (CaM) and proteins present in the CPC and the radial spokes [Wargo et al., 2005; Dymek and Smith, 2007; DiPetrillo and Smith, 2010; Inaba, 2015]. Variations in intracellular pH are also important for ciliary beating [Salathe, 2007]. Bronchial cilia display decreased beat frequency in acidic pH, while alkaline pH produces the opposite effect [Salathe, 2007].

Chlamydomonas and Mouse Mutants

The generation of gene-targeted mice with deficient axonemal components has been critical for the elucidation of differences in phenotypes between mammalian and algal mutants, and in the investigation of numerous ciliary functions, including human pathology [Ibanez-Tallon et al., 2003].

Cpc1/Spef2

Chlamydomonas CPC1 is a 205 kDa protein that possesses a domain with homology to adenylate kinase family members [Zhang and Mitchell, 2004]. The *Cpc1* mutants display flagellar motility defects characterized by reduced beat frequency [Mitchell and Sale, 1999; Zhang and Mitchell, 2004; Mitchell and Smith, 2009]. Ultrastructural studies on isolated axonemes revealed absence of the C1b projection [Mitchell and Sale, 1999; Zhang and Mitchell, 2004; Mitchell and Smith, 2009]. The mammalian orthologue of CPC1, SPEF2 also known as KPL2, is expressed in ciliated cell types and testis [Ostrowski et al., 1999; Sironen et al., 2006; Sironen et al., 2010]. There are several transcripts derived from this gene described in public databases (Tables I and III). *Spef2*-deficient mice develop hydrocephalus, male infertility and sinusitis (Table II). Electron microscopic analysis of epididymal sperm demonstrated absence of recognizable axonemal structures and disorganization of mitochondria and outer dense fibers [Sironen et al., 2011]. Tracheal epithelial cilia beat at lower frequency and possessed a grossly normal 9 + 2 axonemal structure without apparent defects in the dynein arms [Sironen et al., 2011].

Hydin

HYDIN is a polypeptide of >500 KDa encoded by a conserved gene present in the genomes of various protists and metazoans. Although, the transcript encoding the full length protein is well known, other smaller mRNAs have been identified (Tables I and Table III). This protein is a component of the C2b projection [Davy and Robinson, 2003; Lechtreck and Witman, 2007] (Fig. 2). *Hydin* mutant mice develop lethal hydrocephalus with perinatal onset, and early demise before seven weeks of life [Mclone et al., 1971; Raimondi et al., 1976; Davy and Robinson, 2003; Lechtreck et al., 2008] (Table II). Cilia from trachea and ependymal cells lack the C2b central pair projection and have impaired motility with reduced ciliary beat frequency CBF, uncoordinated beat and altered waveform [Lechtreck et al., 2008]. Knockdown of *Hydin* in *Chlamydomonas* results in severe flagellar motility defects [Lechtreck and Witman, 2007]. Flagella also are shorter than those of wild type cells, with some cells lacking flagella completely. Ultrastructural studies revealed axonemes lacking C2b and part of C2c projections [Lechtreck and Witman, 2007]. In humans, recessive *HYDIN* mutations cause primary ciliary dyskinesia (PCD) without randomization of left-right body asymmetry. Respiratory cilia from affected individuals lack the C2b projections as reported in mouse and *Chlamydomonas* models, and show markedly reduced beating amplitudes [Olbrich et al., 2012].

CFAP221/Pcdp1

The novel murine *Pcdp1* gene encodes a 2.5 kb transcript [Lee et al., 2008] and yields a protein ~97 kDa (Pcdp1, primary ciliary dyskinesia protein 1). Mutation of this gene yields several phenotypes commonly associated with primary ciliary dyskinesia. Homozygous mutants on a C57BL/6J background develop severe hydrocephalus and mainly die within the first week of life. However, on other genetic backgrounds (129S6/SvEvTac) mice develop either mild or no hydrocephalus with survival to adulthood (Table II). The male mice are infertile, producing sperm with no visible flagella. Interestingly, the respiratory epithelial cilia have a normal ultrastructure but beat with reduced frequency, and there is an abnormal accumulation of mucus in the mutant sinuses [Lee et al., 2008]. The presence of structurally normal cilia and lack of sperm with mature flagella suggest that the mechanisms regulating the biogenesis of cilia and flagella are likely to be different [Lee, 2011]. FAP221, the orthologue of mammalian Pcdp1, shares 24% amino acid sequence identity when the entire protein sequences are aligned (Table IV). Both Pcdp1 and FAP221 have calmodulin binding sites, and calmodulin preferentially binds to both in the presence of high calcium concentrations [DiPetrillo and Smith, 2010; DiPetrillo and Smith, 2011]. FAP221 together with other proteins (FAP54, FAP46, FAP74 and C1d-87) and calmodulin form a single complex that localizes to the C1d projection [DiPetrillo and Smith, 2010; Brown et al., 2012]. Deficiency of this complex results in severe flagellar motility defects [DiPetrillo and Smith, 2010; DiPetrillo and Smith, 2011; Brown et al., 2012].

CFAP54/Cfap54

The *Chlamydomonas CFAP54* gene encodes a protein of 3,081 amino acids (Table I). This protein is important for the normal assembly of the C1d projection into the axoneme [Brown et al., 2012]. It appears to form a heterotrimeric sub-complex with FAP46 and FAP74 proteins. However, it may directly bind to the C1 central pair microtubule and calmodulin [Brown et al., 2012]. The mouse *Cfap54* gene is located on chromosome 10. There are several transcripts derived from this gene described in public databases (Table I). Recently, *Cfap54* mutant mice were generated and maintained on two different backgrounds. *Cfap54* mutant mice on the C57BL/6J background develop severe hydrocephalus which results in early mortality (Table II). In contrast, mutant mice on mixed C57BL/6J-129S6/SvEvTac background develop only mild hydrocephalus and exhibit early mortality. Males are infertile due to defects in spermatogenesis. Flagellar structures are mostly absent in testicular sperm, and few sperm can be found in the epididymis with most possessing short tails. Respiratory epithelial cilia have reduced beat frequency, which results in perturbed cilia-driven flow. Ultrastructural studies reveal disorganized axonemal microtubules in the few flagella present in the testis. While tracheal epithelial cilia have a 9 + 2 structure, they lack the C1d projection. These findings suggest that CFAP54 is required for assembly and function of mammalian flagella and motile cilia [McKenzie et al., 2015].

PF16/Spag6

The *Chlamydomonas PF16* gene encodes a protein of ~60 kDa, containing eight contiguous 42 amino acid armadillo repeats, which are important for protein-protein interactions. Therefore, the *PF16* gene product has features that promote interactions with other proteins to perform structural or regulatory functions necessary for central microtubule stability and flagellar motility [Smith and Lefebvre, 1997a,b]. *Chlamydomonas pf16* mutants show severe flagellar motility defects and the C1 central microtubule is lost after demembranation of the flagella [Smith and Lefebvre, 1996; Mitchell and Smith, 2009]. The murine orthologue of PF16, SPAG6 also contains eight contiguous armadillo repeats [Sapiro et al., 2000]. Several different transcripts encoding mouse SPAG6 are present in public databases (Tables I and III).

Mice deficient in SPAG6 develop hydrocephalus with early demise of 50% of the animals on a mixed 129/SvJ-C57BL/6J background [Sapiro et al., 2002] (Table II). The surviving mutants are infertile due to functional and structural defects in the sperm flagellar axoneme. The epididymal sperm show a twitching motion, some lack heads and/or have a truncated flagellum and an abnormal midpiece. Ultrastructural studies revealed missing central pairs in the axonemes and disorganized outer dense fibers [Sapiro et al., 2000]. These phenotypes are consistent with the expected function of a protein present in the CPC. However, further characterization of the *Spag6* mutant mice revealed reduced and uncoordinated cilia beat, low cilia density, random basal feet orientation, and reduced epithelial cell polarity [Teves et al., 2014]. Mutation of the *Spag6* gene also results in disorganization of ciliary basal feet in the middle ear, and disrupted planar cell polarity in the middle ear and Eustachian tubes [Li et al., 2014]. Although, these phenotypes may be a consequence of a primary defect in ciliary beating, they may also reflect unique functions of SPAG6 outside of the expected CPC structure, as described below. Studies have shown that SPAG6 is present along the cylindrical outer hair cells (OHCs) in addition to the expected kinocilium localization [Wang et al., 2015]. Its expression in OHC cells appears to be indispensable for the stability and mechanosensory function of these cells [Wang et al., 2015]. Moreover, expression of *Spag6* in Chinese Hamster Ovary (CHO) cells (cells without 9 + 2 axoneme structure) revealed that SPAG6 decorates microtubule structures [Sapiro et al., 2000; Zhang et al., 2002; Zhang et al., 2005]. The association with microtubules and the armadillo repeat domains of SPAG6 suggest that SPAG6 plays a role in the function of tubulin-containing organelles, besides the central pair of microtubules of motile cilia and flagella [Li et al., 2015a,b]. We have recently shown that mutation of the *Spag6* gene disturbs cytoskeleton components and affects cell division, morphology, adhesion, and migration. It also reduced acetylation of α-tubulin [Li et al., 2015a,b], which is one of the more important micro-tubule post-translational modifications. Conversely, overexpression of SPAG6 in CHO and COS-1 cells increases acetylated-tubulin. Similarly, overexpression of *Spag6* in Spag6 mutant mouse embryonic fibroblast (MEFs) increases acetylated-tubulin [Li et al., 2015a,b]. Recognizing that *SPAG6* is a microtubule-associated protein not exclusively confined to the CPC microtubules, these findings may explain a number of the phenotypes observed in Spag6 mutant mouse cells that are not related to tissues with motile cilia. In summary, mammalian SPAG6 may not only regulate ciliary/flagellar motility (functions related to its localization in the CPC), it also plays a role in ciliogenesis, the control of axoneme orientation, cell polarity, division, morphology, adhesion and migration.

A duplication of the *Spag6* gene (*Spag6-BC061194* or *Spag6l*), located on chromosome 2qA3 (chr2:18,620,650-18,672,040), was reported in the mouse and rat [Qiu et al., 2013]. The first characterized *Spag6* mutant mouse had a targeted Spag6 mutation located on chromosome 16. This mutant retains the Spag6-BC061194 gene [Teves et al., 2014]. The amino acid sequences of the two *SPAG6* proteins are 97% identical, but the nucleotide sequences of the two Spag6 genes are significantly different [Teves et al., 2014]. It is not clear yet, whether *Spag6-BC061194* is functional or it is a pseudogene.

PF20/Spag16

The algae *PF20* gene encodes a protein of ~66 kDa which contain five contiguous WD repeats in the carboxyl-terminal half of the protein [Mitchell and Smith, 2009]. These WD repeats are important for interaction with other proteins [Neer et al., 1994]. PF20 also contains regions rich in basic and acidic amino acids as well as alanine-rich regions [Smith and Lefebvre, 1997a,b; Mitchell and Smith, 2009]. PF20 localizes along the length of the C2 microtubule on the intermicrotubule bridges connecting the two CPC microtubules (Fig. 2). The flagella of *Chlamydomonas pf20* mutants have severe motility defects, and upon isolation of flagella and demembranation to prepare axonemes, the mutant axonemes usually lack the entire CPC [Smith and Lefebvre, 1997a,b; Mitchell and Smith, 2009]. The murine orthologue of *pf20* is *Spag16* which encodes two major transcripts of 1.4 and 2.5 kb [Zhang et al., 2002] and yields proteins of 35 (SPAG16S) and 71 (SPAG16L) kDa, respectively [Zhang et al., 2004]. SPAG16L is found in all murine cells with motile cilia and flagella, and SPAG16S is expressed only in male germ cells, where it is predominantly found in specific regions within the nucleus known as nuclear speckles [Zhang et al., 2004; Nagarkatti-Gude et al., 2011]. The 71 kDa isoform is the orthologue of PF20 and contains a coiled-coil region followed by up to seven contiguous WD repeats in the C-terminus [Zhang et al., 2004; Zhang et al., 2007a,2007b; Nagarkatti-Gude et al., 2011]. Several CPC proteins were shown to interact with SPAG16. SPAG6 co-immunoprecipitates with SPAG16 in extracts of CHO cells expressing both proteins [Zhang et al., 2002]. Similarly, the kinase, Stk36, physically associates with SPAG16 after expression of both proteins in HEK 293T cells [Wilson et al., 2009; Nozawa et al., 2013]. However, Stk36 does not interact with SPAG6 [Wilson et al., 2009]. Recently, it has been shown that the manchette (a unique organelle involved in the assembly of the sperm flagella) localization of SPAG16L in elongating spermatids is dependent upon MEIG1 and PACRG proteins [Li et al., 2015a,b]. Targeted disruption of *Spag16* exon 11 affects the expression of both SPAG16L and SPAG16S, and results in spermatogenesis defects in chimeric mice [Zhang et al., 2004]. In contrast, a targeted mutation that deletes exon 2 and 3 of the *Spag16* gene affects only SPAG16L expression. The resulting null mice show no evidence of primary ciliary dyskinesia phenotypes including hydrocephalus, or sinusitis, [Zhang et al., 2006], and display tracheal epithelial cells with motile cilia [Teves et al., 2013]. Males have normal spermatogenesis but are infertile due to severe sperm motility defects, even though the sperm have a normal axoneme ultrastructure [Zhang et al., 2006] (Table II).

Functional studies have been carried out on mouse sperm lacking SPAG16L [Lesich et al., 2010]. Spag16L-null sperm exhibited much less bending of the flagellum during the beat, and the amount of microtubule sliding in the reverse bend direction was selectively restricted. This suggests that there is limited activation of the dyneins on one side of the axoneme. Additionally, the flagellar response to calcium was greatly reduced in this model. This response requires activation of the dyneins on specific outer microtubule doublets; the same dyneins required for reverse bend formation. In axonemes prepared to disintegrate in vitro as a result of microtubule sliding, little or no extrusion of specific microtubule doublets was observed, a finding consistent with reduced activity of their dyneins. This deficit in motor function can explain the observed motility characteristics of the Spag16L-null sperm [Lesich et al., 2010].

A heterozygous mutation disrupting the human *SPAG16* gene results in biochemical instability of the interacting proteins of the central apparatus in response to a thermal challenge [Zhang et al., 2007a,b]. Freezing-boiling of isolated sperm from two male heterozygotes for the *SPAG16* mutation, resulted in the loss of the SPAG16L protein, SPAG6, and the 28-kDa fragment of SPAG17 [Zhang et al., 2007a,2007b]. Ultrastructure of sperm from these subjects revealed axonemes that are missing the central pair, and the outer double microtubules [Zhang et al., 2007a,b].

Double knockouts of *Spag6* and *Spag16* have been created revealing synergistic interactions of the two genes on cilia-related phenotypes [Zhang et al., 2007a,b]. The double mutant mice displayed more profound growth retardation and hydrocephalus compared to mice nullizygous for SPAG6. They also died younger, and mortality was significantly greater. The double mutant mice developed pneumonia and its complications, including hemorrhage, edema, and atelectasis [Zhang et al., 2007a,b]. These lung pathologies were not observed in mice nullizygous for a mutation in either single gene. In addition, the ultrastructure of cilia in both brain and lung of the double mutant mice appeared to be grossly normal [Zhang et al., 2007a,b].

PF6/Spag17

The Chlamydomonas *PF6* gene encodes a large polypeptide (2301 amino acids) that contains alanine-rich (18%), proline-rich (12%), and highly basic domains [Mitchell and Smith, 2009]. Domains near the C-terminus of PF6 are essential for flagellar motility and assembly of C1a projection [Goduti and Smith, 2012]. Meanwhile, the N-terminal half is not required for the assembly but it is important for stability of the C1a complex [Goduti and Smith, 2012]. Flagella from *pf6* mutants twitch ineffectively, and lack the C1a projection, and some polypeptides that co-sediment and co-immunoprecipitate with PF6 protein [Rupp et al., 2001; Mitchell and Smith, 2009]. SPAG17 is the mammalian orthologue of PF6. The murine *Spag17* gene encodes a full-length 250 kDa protein found in testis and tissues with motile cilia, and a 97 kDa protein present in testis that derived from an alternatively spliced variant. The 97 kDa protein is cleaved to generate 72 kDa (N-terminus) and 28 kDa (C-terminus) fragments in epididymal and ejaculated sperm [Zhang et al., 2005]. Moreover, there is a maturation-dependent pattern of SPAG17 isoform expression in fractionated germ cells with the 97 kDa isoform appearing prior to the 250 kDa isoform, and the appearance of 72 and 28 kDa fragments of the 97 kDa isoform in epididymal sperm. The 28 kDa SPAG17 isoform is missing in SPAG6-deficient mice, supporting the hypothesis that the C-terminus of the 97 kDa SPAG17 isoform, of which the 28 kDa form is a fragment, is the interacting partner with SPAG6 in the CPC [Zhang et al., 2005]. *Spag17*-null mice have an expected primary ciliary dyskinesia phenotype characterized by immotile nasal and tracheal cilia, reduced clearance of nasal mucus, profound respiratory distress associated with lung fluid accumulation and disruption of the alveolar epithelium, hydrocephalus, and neonatal demise within 12 hours of birth (Table II). Electron microscopy analysis revealed the loss of one CP microtubule in approximately one quarter of tracheal cilia axonemes, the absence of a C1 microtubule projection, and other less frequent CPC structural abnormalities [Teves et al., 2013]. However, further characterization of *Spag17* mutant revealed unexpected functions of *Spag17* in regulation of skeletal growth and mineralization. Mutants show shorter hind limbs due to increased bone formation and mineralization in the femur, and reduced growth plate and bone formation in the tibia. Other skeletal malformations include fused sternebrae, reduced mineralization in the skull, medial and metacarpal phalanges. Primary cilia from chondrocytes, osteoblasts, and embryonic fibroblasts (MEFs) are shorter and fewer cells have primary cilia in comparison to cells isolated from wild-type mice [Teves et al., 2015]. Interestingly, an antibody against the C-terminal domain of SPAG17 detected a ~75 kDa protein expressed in MEFs, suggesting that it is an isoform of the 250 kDa full length protein. This ~75 kDa protein may be derived from alternative splicing or post-translational processing. These findings reveal an unexpected expression pattern and function of the *Spag17* gene. In addition, they point out several differences between the mammalian gene and its orthologue in algae. Interestingly, single-nucleotide polymorphisms (SNPs) in the *SPAG17* gene have been associated with human height [Weedon and Frayling, 2008; Weedon et al., 2008; Takeuchi et al., 2009; Zhao et al., 2010; N'Diaye et al., 2011; van der Valk et al., 2014; Wood et al., 2014] and idiopathic short stature in a Korean population [Kim et al., 2010]. These data are consistent with the shorter hind limbs observed in *Spag17* mutant mice [Teves et al., 2015].

Radial Spoke Mutations

The radial spokes play an essential role in the control of dynein arm activity by transmitting signals from the central pair complex to the dynein arms. Defects in the CPC, including loss of one or both CPC microtubule have been reported in families with mutations that disrupt genes encoding radial spoke head (RSPH) proteins [Castleman et al., 2009]. The abnormalities in the CPC in individuals with mutations in the radial spoke head genes *RSPH1* [Kott et al., 2013; Onoufriadis et al., 2014], *RSPH3* [Jeanson et al., 2015], *RSPH9* and *RSPH4A* [Castleman et al., 2009; Zie˛tkiewicz et al., 2012] suggest that the nexus between the CPC and radial spokes is critical for stabilizing the human cilia axoneme, as in *Chlamydomonas* [Mitchell and Smith, 2009].

Positive Selection of CPC Genes

The positive and negative selection of genes has played a key role in the generation of the enormous diversity of species observed in the nature. Mutations in coding regions of genes that change the resulting amino acid sequence of the protein have been positively selected and there are numerous studies documenting positive selection in a variety of genes and organisms, including immune-response-related genes, viral genes, genes involved in reproduction, and genes related to sensory perception and olfaction in humans [Nielsen, 2005]. Evidence suggests that genes involved in reproduction are evolving rapidly, especially proteins present in the sperm [Torgerson et al., 2002; Vicens et al., 2014]. Several CPC proteins were shown to be positively selected in mammals. For example, *SPAG17* was reported to be one of a small number of genes that distinguishes humans from Neanderthals [Green et al., 2010], and its encoding protein was suggested to be positively selected [Torgerson et al., 2002]. Nucleotide substitutions were detected at amino acid positions 431 (humans have a Tyr and Neanderthals and mice have a Asp), and 1415 (humans have a Thr, Neanderthals have an Ala and mice have a Lys) [Green et al., 2010]. *SPAG6* is another CPC gene that has undergone positive selection [Williamson et al., 2007], although its selection is more likely to be related to transcriptional control than to a change in the protein sequence [Hu et al., 2012]. As mentioned earlier, the *Spag6* gene was recently discovered to be duplicated in rodents. The newly identified, highly divergent *Spag6*-BC061194 gene is the parental gene [Qiu et al., 2013]. It has been proposed that the *Spag6* gene represents a rare exception from the general rule of rapid evolution of derived rather than parental genes following gene duplication [Qiu et al., 2013].

Central Pair Complex Isoforms

The orthologous CPC proteins present in mouse and *Chlamydomonas* have variable amino acid sequence identity and similarity that presumably reflect conserved functions as well as evolving roles for these molecules (Table IV). SPAG6 is highly conserved whereas SPAG17 shows the least amino acid sequence similarity and identity. In mice and humans, the CPC genes code for a variety of isoforms that are generated by either an alternative promoter, alternative splicing, or post-translational processing [Zhang et al., 2002; Zhang et al., 2004; Zhang et al., 2006; Nagarkatti-Gude et al., 2011] (Table I and III). The expression of isoforms appears to be tissue/cell specific. Although most of the different isoforms were cloned from the same tissues, some of the isoforms appear to be present only in one tissue (e.g., in *mus musculus*, Spag17.eSep07 and Spag17.fSep07 are only present in the cerebellum; in *homo sapiens* Spag6.nAug10 is only present in the eye) (Table III). In addition, some of the isoforms appear to be putative proteins (e.g., in *homo sapiens* Spag6.kAug10; Spag16.lAug10 and Spag16.sAug10; and Spag17.hAug10, Spag17.lAug10, Spag17.mAug10 and Spag17.nAug10 were not observed in any tissue). The fact that these genes encode several isoforms allows for greater complexity in expression pattern and function. However, little is known about the function of these protein isoforms. Most of our knowledge comes from mutants where the full length protein isoform have been disrupted. The exceptions are SPAG17 and SPAG16, where different isoforms or truncated proteins appear to be related to different tissue/cell functions [Zhang et al., 2005; Nagarkatti-Gude et al., 2011; Teves et al., 2015].

Tissue/cell-Specific Roles

Defects in cilia or flagella motility/function caused by mutations in CPC genes appear to depend upon the cellular context and genetic background in mammals. For instance, the presence of structurally normal cilia and lack of sperm with mature flagella in the *Pcdp1* knockout mice suggests that the mechanisms regulating the biogenesis of motile cilia and flagella are different [Lee, 2011]. As noted above, *Spef2* deficient mice have tracheal epithelial cilia with a normal 9 + 2 axonemal structure without apparent defects in the dynein arms, but epididymal sperm lack recognizable axonemal structures [Sironen et al., 2011]. SPAG16L-null mice show no evidence of cilia dysfunction such as hydrocephalus, sinusitis, and bronchial infection [Zhang et al., 2006], and have tracheal epithelial cells with motile cilia [Teves et al., 2013]. However, males are infertile due to severe sperm motility defects, even though the sperm have a normal axoneme ultrastructure [Zhang et al., 2006]. In the *Spag17* mutant mouse, the rapid neonatal demise is associated with a profound respiratory phenotype characterized by immotile cilia that is not observed in knockouts of the *Spag6* and *Spag16* genes [Teves et al., 2013]. Although the genetic background of mutant mice may significantly influence the phenotypes in knockout mice, the observations summarized above suggest that due to the complexity of sperm flagellar development, the assembly of the sperm flagellar axoneme may be particularly sensitive to mutations in genes encoding axonemal proteins.

Although, the CPC *Spag* genes were initially analyzed in the context of male reproduction, new studies suggest that these genes play unique roles in different cell types. Overexpression of *SPAG6* was identified in acute myeloid leukemia compared to healthy bone marrow [Steinbach et al., 2006]. The levels of expression of this gene have been used to assess minimal residual disease [Steinbach et al., 2015]. In addition, SPAG6 has been shown to be important for microtubule stability in MEFs[Li et al., 2015a,b]. SPAG6 also appears to control neuronal migration as well as neurite branching and elongation in mice [Yan et al., 2015]. Expression of *SPAG16* in synovium and fibroblast-like synoviocytes has been suggested to protect against joint destruction in autoantibody-positive rheumatoid arthritis [Knevel et al., 2014]. This gene is also associated with bipolar disorder in Canadian and UK populations [Xu et al., 2014]. In addition, *SPAG16* is reported to be upregulated in astrocytes in multiple sclerosis patients and encephalo-myelitis spinal cord lesions [de Bock et al., 2014]. Expression of *SPAG16L*, *SPAG6* and *SPAG17* is associated with several cancers [Silina et al., 2011]. However, the seemingly abnormal expression of these genes needs to be further studied to determine whether these observations are related to novel functions and disease pathophysiology. On the other hand, expression of *Spag17* by mouse chondrocytes and osteoblasts appears to play a role in skeletal development, linear growth and bone mineralization [Teves et al., 2015]. This extends the role of *Spag17* gene outside tissues with motile 9 + 2 cilia. It is not clear yet if this new function is directly associated to bone and cartilage physiology or it is mediated through defects in development and function of primary cilia, or a combination of both.

Conclusions

Ciliary and flagellar genes, proteins and functions are believed to be conserved throughout evolution from protists to humans [Ibanez-Tallon et al., 2003; Mitchell, 2004; Carvalho-Santos et al., 2011]. Most of the studies of CPC proteins have been performed in *Chlamydomonas*. However, during the past decade, the field of ciliary/flagellar biology has grown tremendously due to advances in technology and gene-targeted models. These studies have pointed out significant differences in the roles of the CPC proteins in *Chlamydomonas* and mammals, including variability in the architecture, function, and biogenesis of the axoneme components [Wanner et al., 1996; Silflow and Lefebvre, 2001; Carvalho-Santos et al., 2011]. Evidence also suggests that in mammals, the CPC proteins (SPAG6, SPAG16 and SPAG17) have functions outside of the CPC. They also interact with each other [Zhang et al., 2002; Zhang et al., 2005] and might work as a complex. These interactions have not been identified or evaluated in *Chlamydomonas* [Neilson et al., 1999; Sapiro et al., 2000; Sapiro et al., 2002; Zhang et al., 2002; Zhang et al., 2005]. To date, no evidence for functions of SPEF2, HYDIN, Pcdp1 and CFAP54 outside the CPC have been reported. In *Chlamydomonas*, the CPC genes encoding orthologues of mammalian proteins yield only a single protein product, while in mice and humans, these genes encode a variety of isoforms that are generated either by an alternative promoter, alternative splicing, or post-translational processing [Zhang et al., 2002; Zhang et al., 2004; Zhang et al., 2006; Nagarkatti-Gude et al., 2011]. However, little is known about these protein isoforms, and further studies are required to determine whether they have distinct functional roles. That being said, we do have clear indication that different isoforms of SPAG17 and SPAG16, have different tissue/cellular locations than their full length sisters and different functions [Zhang et al., 2005; Nagarkatti-Gude et al., 2011; Teves et al., 2015]. The greater complexity of mammalian CPC proteins and functions provides a foundation for the elucidation of the mechanisms underlying ciliary/flagellar motility, and a better understanding of the molecular and structural basis of the genetically and phenotypically heterogeneous disorder of primary ciliary dyskinesia.

Acknowledgments

DRN-G was supported by National Institutes of Health-NICHD grant 5F31HD062314. ZZ is supported by National Institutes of Health grant R01HD076257. JFS was supported by a National Institutes of Health-NICHD grant R01HD037416.

Abbreviations used

ARM	armadillo
CaM	calmodulin
CBF	Ciliary beat frequency
CHO	Chinese hamster ovary
CPC	central pair complex
PCD	primary ciliary dyskinesia
RSPH	radial spoke head

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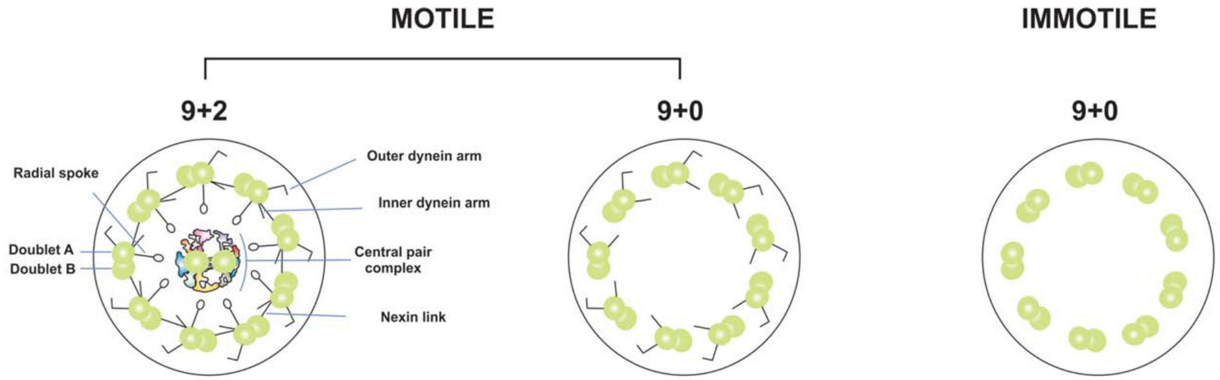

Location	Pathology
Respiratory epithelial cells	Bronchiectasis, bronchitis, sinusitis
Ependymal cells	Hydrocephalus
Middle ear	Otitis media
Oviductal epithelium	Reduced fertility
Sperm	Infertility

Location	Pathology
Node	Situs inversus

Location	Pathology
Kidney, pancreas and liver	Cystic disorders
Skeleton	Osteochondro-dysplasia
Eye	Retinitis pigmentosa
Embryo	Developmental deffects

Fig. 1. Axoneme structures of the different types of cilia

Cilia and flagella are specialized organelles involved in motility. The structure of both is based on nine pairs of microtubules surrounding two single microtubules, which form the central pair complex in motile cilia. Attached to the nine microtubules are other structures, such as the inner and outer dynein arms, the radial spokes and nexin links. Cilia located in the embryonic node (the nodal cilia) lack the central pair complex but maintain the dynein arms, which confer the capacity for rotary movement. Immotile cilia or primary cilia lack the central pair complex and the other structures around the nine pair of outer microtubules. They are present in almost all vertebrate cells. Ciliopathies encompass a variety of distinct disorders depending upon the organs or tissues affected. The main disorders resulting from defects in the development or function of cilia.

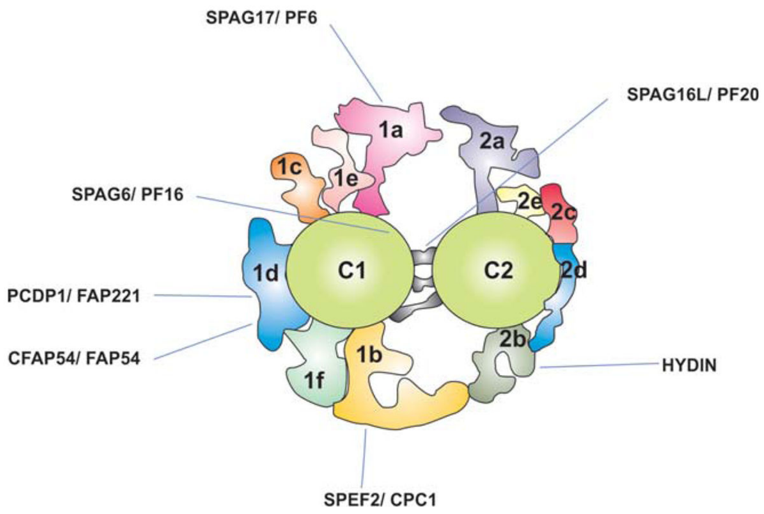

Fig. 2.

Schematic representation of the central pair complex based on the cross section of a *Chlamydomonas* axoneme obtained by cryoelectron tomography. The location of selected CPC proteins is indicated.

Table I

Transcripts Encoding Humans, Mouse, and *Chlamydomonas* CPC Proteins

Gene	Specie	Transcript ID	Protein coding	Length (aa)
Spef2/cpc1	*Homo sapiens*	ENST00000356031	Yes	1822
		ENST00000282469	Yes	514
		ENST00000440995	Yes	1818
		ENST00000509059	Yes	943
		ENST00000303129	Yes	619
		ENST00000504054	Yes	389
		ENST00000510777	Yes	240
		ENST00000506526	Yes	151
		ENST00000513078	Yes	115
		ENST00000505847	Yes	145
		ENST00000508817	Yes	115
		ENST00000506759	Yes	120
		ENST00000503074	Non-coding	-
		ENST00000505088	Non-coding	-
		ENST00000502454	Non-coding	-
	Mus musculus	ENSMUST00000041840	Yes	875
		ENSMUST00000160236	Yes	1724
		ENSMUST00000159288	Yes	1734
		ENSMUST00000159093	Yes	622
		ENSMUST00000162780	Yes	513
		ENSMUST00000159368	Yes	456
	Chlamydomonas reinhardtii	EDO96779	Yes	1929
Hydin	*Homo sapiens*	ENST00000393567	Yes	5121
		ENST00000321489	Yes	1017
		ENST00000538248	Yes	950
		ENST00000541601	Yes	940
		ENST00000288168	Yes	725
		ENST00000538382	Yes	204
		ENST00000545267	Yes	36
		ENST00000539973	Yes	39
		ENST00000542890	Yes	127
		ENST00000316490	Yes	5120
		ENST00000393550	Yes	740
		ENST00000448089	Yes	1214
		ENST00000448691	Yes	1017
		ENST00000539447	Yes	151
		ENST00000545230	Yes	528
		ENST00000393552	Yes	833
		ENST00000378856	Yes	49

Gene	Specie	Transcript ID	Protein coding	Length (aa)
		ENST00000538568	Yes	113
		ENST00000546257	Yes	43
		ENST00000543639	Non-coding	-
		ENST00000542283	Non-coding	-
		ENST00000540892	Non-coding	-
		ENST00000543521	Non-coding	-
		ENST00000309900	Non-coding	-
		ENST00000537789	Non-coding	-
		ENST00000536785	Non-coding	-
		ENST00000546297	Non-coding	-
	Mus musculus	ENSMUST00000043141	Yes	5154
	Chlamydomonas reinhardtii	EDP09735	Yes	4524
Pcdp1/CFAP221	*Homo sapiens*	ENST00000602047	Yes	554
		ENST00000594371	Yes	173
		ENST00000442513	Yes	175
		ENST00000594141	Yes	46
		ENST00000599827	Yes	118
		ENST00000434869	Yes	96
		ENST00000413369	Yes	840
		ENST00000598644	Yes	187
		ENST00000443972	Yes	399
		ENST00000295220	Yes	269
		ENST00000413057	Yes	53
		ENST00000600951	Yes	88
		ENST00000594033	Yes	90
		ENST00000463985	Yes	134
		ENST00000475569	Non-coding	-
		ENST00000488358	Non-coding	-
		ENST00000464578	Non-coding	-
		ENST00000597189	Non-coding	-
	Mus musculus	ENSMUST00000174370	Yes	836
		ENSMUST00000037840	Yes	836
		ENSMUST00000174458	Yes	178
	Chlamydomonas reinhardtii	EDP00231	Yes	645
Cfap54/CFAP54	*Homo sapiens*	ENST00000524981	Yes	3096
		ENST00000550977	Yes	265
		ENST00000553778	Yes	644
		ENST00000554108	Non-coding	-
		ENST00000556591	Non-coding	-
		ENST00000342887	Non-coding	-
		ENST00000554621	Non-coding	-
		ENST00000554821	Non-coding	-

Gene	Specie	Transcript ID	Protein coding	Length (aa)
	Mus musculus	ENSMUST00000168110	Yes	3106
		ENSMUST00000020200	Yes	816
		ENSMUST00000168617	Yes	598
		ENSMUST00000164979	Yes	314
		ENSMUST00000163209	Yes	187
		ENSMUST00000170065	Yes	167
		ENSMUST00000067705	Non-coding	-
		ENSMUST00000165090	Non-coding	-
		ENSMUST00000171781	Non-coding	-
		ENSMUST00000168080	Non-coding	-
	Chlamydomonas reinhardtii	EDP00642	Yes	3081
Spag6/Pf16	*Homo sapiens*	ENST00000313311	Yes	458
		ENST00000376601	Yes	270
		ENST00000376603	Yes	585
		ENST00000376624	Yes	509
		ENST00000435326	Yes	246
		ENST00000456231	Yes	191
		ENST00000538630	Yes	484
		ENST00000488555	Non-coding	-
		ENST00000487973	Non-coding	-
		ENST00000490361	Non-coding	-
	Mus musculus	ENSMUST00000023468	Yes	507
		ENSMUST00000154624	Non-coding	-
		ENSMUST00000132033	Non-coding	-
		ENSMUST00000125017	Non-coding	-
		ENSMUST00000147539	Non-coding	-
		ENSMUST00000151132	Non-coding	-
		ENSMUST00000141835	Non-coding	-
	Chlamydomonas reinhardtii	EDP02264	Yes	475
Spag16/Pf20	*Homo sapiens*	ENST00000272898	Yes	347
		ENST00000331683	Yes	631
		ENST00000374309	Yes	537
		ENST00000413312	Yes	251
		ENST00000447990	Yes	374
		ENST00000432529	Yes	183
		ENST00000451561	Yes	255
		ENST00000406979	Yes	57
		ENST00000452556	Yes	64
		ENST00000440779	Yes	94
		ENST00000420497	Yes	98
		ENST00000480494	Non-coding	-
		ENST00000414961	Non-coding	-

Gene	Specie	Transcript ID	Protein coding	Length (aa)
	Mus musculus	ENSMUST00000065425	Yes	639
		ENSMUST00000113940	Yes	405
		ENSMUST00000161131	Yes	253
		ENSMUST00000161310	Non-coding	
	Chlamydomonas reinhardtii	EDP04185	Yes	521
Spag17/Pf6	*Homo sapiens*	ENST00000336338	Yes	2223
		ENST00000437255	Yes	660
		ENST00000465053	Non-coding	-
		ENST00000463628	Non-coding	-
		ENST00000477444	Non-coding	-
		ENST00000473472	Non-coding	-
		ENST00000486589	Non-coding	-
		ENST00000470550	Non-coding	-
		ENST00000483383	Non-coding	-
		ENST00000492438	Non-coding	-
		ENST00000469128	Non-coding	-
		ENST00000466857	Non-coding	-
		ENST00000478697	Non-coding	-
	Mus musculus	ENSMUST00000164539	Yes	2320
		ENSMUST00000127518	Non-coding	-
		ENSMUST00000152586	Non-coding	-
		ENSMUST00000143050	Non-coding	-
	Chlamydomonas reinhardtii	EDP06248	Yes	2301

Data were extracted from Ensembl.

Table II

Phenotypes of CPC Gene Mutations in Mice

Gene	Survival	Airway cilia motility	Hydrocephalus	Sperm motility	Male fertility	Female fertility	Mouse background	References
Spef2	Variable, some animals died within a few days after birth and a small number lived longer than 50 days	Reduced CBF	Yes	?	Infertile	?	C57BL/6J	Sironen et al., 2011
	Survival to adulthood	?	No	short tail and abnormal flagellar shape	Infertile	?	mixed C57BL/6J-129S6/SvEvTac	
Hydin	Mostly death by 3 weeks after birth	Reduced CBF Impaired ciliary bending	Yes	?	?	?	FVB/N	Mclone et al., 1971 Raimondi et al., 1976 Davy and Robinson, 2003 Lechtreck et al., 2008
Pcdp1	Frequently death within 1 week after birth	?	Yes	?	?	?	C57BL/6J	Lee et al., 2008
	Survival to adulthood	Reduced CBF	Mild or No	No mature sperm	Infertile	?	mixed C57BL/6J-129S6/SvEvTac	
Cfap54	Early mortality	?	Yes	?	?	?	C57BL/6J	McKenzie et al.,2015
	Normal	Reduced CBF and cilia-driven flow	Mild	Only few sperm in the epididymis with short tail	Infertile		mixed C57BL/6J-129S6/SvEvTac	
Spag6	50% survival to adulthood	Reduced CBF Uncoordinated beat	Yes	Quaking motion	Infertile	Normal	mixed 129/SvJ- C57BL/6J	Sapiro et al., 2000 Sapiro et al., 2002 Teves et al., 2014
Spag16	Normal	Grossly normal	No	Quaking motion	Infertile	Normal	mixed 129/SvJ- C57BL/6J	Zhang et al., 2002 Zhang et al., 2004
Spag17	Neonatal Death	Mostly immotile Some uncoordinated beat	Yes	?	?	?	mixed C57BL/6J-129S4/SvJ	Teves et al., 2013

Table III

Human and Mouse SPAG Protein Isoforms

Specie	Gene	mRNA variant	Predicted protein MW (kDa)	pI	Tissue
Homo sapiens	*SPEF2*	Spef2.aAug10	209.8	6.1	Lung, uterus, breast carcinoma, brain, amygdala, other tissues
		Spef2.bAug10	151.0	5.6	Testis, placenta
		Spef2.cAug10	108.5	6.7	Amygdala, uterus, breast cancer, eyeball, hippocampus,other tissues
		Spef2.dAug10	100.2	7.1	Testis
		Spef2.eAug10	70.8	6.3	Lung
		Spef2.fAug10	61.2	8.3	Testis, brain, bladder, other tissues
		Spef2.gAug10	46.8	6.2	Spinal cord
		Spef2.hAug10	27.4	9.0	Brain, embryonic stem cells
		Spef2.iAug10	16.5	8.8	Hippocampus
		Spef2.jAug10	16.0	8.5	Intestine
		Spef2.kAug10	12.9	9.1	Lung
		Spef2.lAug10	13.5	9.5	Eye
		Spef2.mAug10	12.8	9.1	Kidney
		Spef2.nAug10	10.9	7.8	Uterus, fetal heart, pooled melanocyte, pooled tumors
		Spef2.oAug10	15.8	8.0	Hippocampus, thalamus
		Spef2.pAug10	9.4	6.4	Testis
		Spef2.qAug10- unspliced	6.2	9.6	Embryonic stem cell
Homo sapiens	*HYDIN*	Hydin.aAug10	575.8	6.3	Testis, brain, lung, uterus, other tissues
		Hydin.bAug10	123.1	6.8	Testis
		Hydin.cAug10	114.2	6.5	Testis
		Hydin.dAug10	114.2	6.5	
		Hydin.eAug10	106.3	6.2	Testis, brain, amygdala
		Hydin.fAug10	105.3	6.2	Testis, hippocampus, pancreas
		Hydin.gAug10	86.6	6.1	Testis, head, pancreas
		Hydin.hAug10	81.6	6.6	Testis, lung
		Hydin.iAug10	26.9	7.9	Pancreas, T cells, head, kidney
		Hydin.jAug10	25.0	5.8	Eye
		Hydin.kAug10	21.5	8.1	Amygdala

Specie	Gene	mRNA variant	Predicted protein MW (kDa)	pI	Tissue
		Hydin.lAug10	15.8	5.2	
		Hydin.mAug 10-unspliced	14.9	5.5	Carcinoid, lung, head, pancreas, kidney, parathyroid
		Hydin.nAug10	7.3	7.5	
		Hydin.oAug10	26.0	5.5	Testis, trachea
		Hydin.pAug10-unspliced	10.1	6.7	Eye
		Hydin.qAug10	9.2	9.1	
		Hydin.rAug10-unspliced	3.9	8.9	Testis
		Hydin.sAug10-unspliced	3.2	10.7	Eye
		Hydin.tAug10-unspliced	3.3	11.5	Skeletal muscle
		Hydin.uAug10-unspliced	4.2	6.8	Head
		Hydin.vaAug10-unspliced	4.9	9.1	Placenta
Homo sapiens	*PCDP1*	Pcdp1.aAug10	64.4	8.0	Testis, lung, trachea, uterus, cerebellum, other tissues
		Pcdp1.bAug10	60.2	8.3	Lung, kidney, cerebellum, heart, pulled tumors, other tissues
		Pcdp1.cAug10	34.9	9.0	Liver and spleen
		Pcdp1.dAug10	21.4	8.1	Trachea
		Pcdp1.eAug10	17.2	8.8	Amygdala, embryonic stem cells, hyppocampus
		Pcdp1.fAug10	13.2	5.9	Brain
		Pcdp1.gAug10	13.5	5.9	
		Pcdp1.hAug10	9.6	5.8	Kidney
		Pcdp1.iAug10	8.5	5.7	pooled tumors from uterus, endometrial adenocarcinoma
		Pcdp1.jAug10-unspliced	2.6	8.0	Testis, brain, blood, carcinoid, eye, heart, other tissues
		Pcdp1.kAug10	5.9	9.4	Brain
		Pcdp1.lAug10	3.6	4.0	Carcinoid, lung
		Pcdp1.mAug10-unspliced	4.7	9.2	
Homo sapiens	*SPAG6*	Spag6.aAug10	55.5	6.9	Testis, lung, pooled germ cell tumors, pancreas, islets of langerhans, other tissues.
		Spag6.bAug10	52.4	7.0	Testis
		Spag6.cAug10	52.5	6.7	Testis
		Spag6.dAug10	49.7	6.8	Testis, brain, medulla, hippo- campus, trachea, corpus callosum, hypothalamus, other tissue
		Spag6.eAug10	38.0	8.9	Lung, mucoepidermoid carcinoma, brain, carcinoid, hippocampus
		Spag6.fAug10	30.6	9.9	Brain, medulla
		Spag6.gAug10	28.7	8.3	Brain, medulla

Specie	Gene	mRNA variant	Predicted protein MW (kDa)	pI	Tissue
		Spag6.hAug10	23.1	7.2	Pooled germ cell tumors, pooled lung and spleen
		Spag6.iAug10	22.6	11.4	Hippocampus, substantia nigra
		Spag6.jAug10	22.6	8.6	Insulinoma, pancreas, brain
		Spag6.kAug10	17.6	6.2	
		Spag6.lAug10	9.3	9.3	Lung from mucoepidermoid carcinoma
		Spag6.mAug10	7.6	8.6	Testis
		Spag6.nAug10	4.1	7.0	Eye
Homo sapiens	*SPAG16*	Spag16.aAug10	70.8	6.4	Testis, brain, hypothalamus, myeloma, pooled colon, kidney, stomach, trachea, embryonic stem, other tissues
		Spag16.bAug10	65.2	6.9	Breast carcinoma, hippocampus, insulinoma, pancreas,testis, brain, hippocampus,other tissues
		Spag16.cAug10	61.2	8.3	Testis, lung, spleen
		Spag16.dAug10	50.7	8.4	Testis, pancreas
		Spag16.eAug10	42.8	6.9	Trachea
		Spag16.fAug10	29.1	8.2	Germinal center B cell, hippocampus
		Spag16.gAug10	23.6	9.3	Brain, testis
		Spag16.hAug10	21.3	5.4	Brain
		Spag16.iAug10	20.6	4.3	Lung, germinal center B cell, kidney, pancreas, prostate,brain, neuroblastoma, other tissues
		Spag16.jAug10	21.4	10.3	Hippocampus
		Spag16.kAug10	20.3	5.7	Pooled germ cell tumors
		Spag16.lAug10	17.1	5.8	
		Spag16.mAug10	16.2	6.1	Insulinoma from pancreas
		Spag16.nAug10	11.8	5.8	
		Spag16.oAug10	12.5	8.1	Liver, spleen
		Spag16.pAug10	11.9	5.7	Colon, colon tumor, RER + , eyeball
		Spag16.qAug10	9.2	9.7	Pancreas
		Spag16.rAug10-unspliced	6.0	9.2	Kidney
		Spag16.sAug10-unspliced	3.2	7.7	
		Spag16.tAug10	2.9	7.4	Testis
		Spag16.uAug10-unspliced	2.8	4.6	Gall bladder
		Spag16.vaAug10-unspliced	3.9	3.3	Uterus tumor
Homo sapiens	*SPAG17*	Spag17.aAug10	251.7	6.6	Testis, lung, breast, breast carcinoma, carcinoid, pooled-skin, other tissues
		Spag17.bAug10	128.9	6.2	Kidney tumor, moderately- differentiated endometrial adenocarcinoma, testis, uterus

Specie	Gene	mRNA variant	Predicted protein MW (kDa)	pI	Tissue
		Spag17.cAug10	121.0	6.5	Trachea
		Spag17.dAug10	68.4	6.9	Trachea, eye
		Spag17.eAug10	39.7	8.3	Lung
		Spag17.fAug10	31.0	5.0	Lung
		Spag17.gAug10	27.2	8.2	Lung
		Spag17.hAug10	20.3	8.4	
		Spag17.iAug10-unspliced	14.0	8.8	Lung
		Spag17.jAug10	13.7	8.9	Liver, spleen
		Spag17.kAug10	11.9	6.3	Liver, spleen
		Spag17.lAug10	11.6	9.3	
		Spag17.mAug10	8.7	8.5	
		Spag17.nAug10	7.5	8.6	
		Spag17.oAug10-unspliced	4.9	6.5	Stomach
		Spag17.pAug10-unspliced	6.5	10.3	Pooled skin
		Spag17.qAug10-unspliced	5.0	8.0	Placenta
		Spag17.rAug10	11.6	9.3	Kidney
		Spag17.sAug10	9.2	3.8	Lung
Mus musculus	*Spef2 (*C230086A09Rik*)*	Spef2.aSep07	100.1	7.6	Testis, testicles, brain, thymus, kidney, lung, spleen, liver, heart, ovary, embryo
		Spef2.bSep07	53.9	8.4	Testis, thymus, cerebellum
		Spef2.cSep07	21.9	8.9	Testis
		Spef2.dSep07	13.4	6.4	
		Spef2.eSep07-unspliced	10.1	9.6	Cerebellum, endoderm, rathke’s pouches
Mus musculus	*Hydin*	Hydin.aSep07	581.5	6.6	Testis, testicles, 18-day preleptotene spermatocytes, brain,ovary
		Hydin.bSep07	150.0	6.5	Lung
		Hydin.cSep07-unspliced	54.9	6.6	Cortex, whole body
		Hydin.dSep07	8.9	12.1	Testis, unfertilized egg
		Hydin.eSep07-unspliced	7.1	6.7	
		Hydin.fSep07	10.3	7.6	Testis
		Hydin.gSep07-unspliced	6.6	10.5	
Mus musculus	*Pcdp1 (*Gm101*)*	Pcdp1.aSep07	45.9	7.4	Testis, 18-day preleptotene spermatocytes, round spermatids, mammary gland
Mus musculus	*Spag6*	Spag6.aSep07	55.3	6.8	Testis, cerebellum, spinal cord, visual cortex, hypothalamus, corpora quadrigemina, cortex, oviduct, eye

Specie	Gene	mRNA variant	Predicted protein MW (kDa)	pI	Tissue
		Spag6.bSep07	47.4	6.7	Testis, 18-day leptotene and zygotene spermatocytes
		Spag6.cSep07-unspliced	18.1	10.9	Testis, tongue, embryo, liver, pancreas, hippocampus, small intestine, blood, carcinoma, other tissues
		Spag6.dSep07	15.7	7.9	Stomach
		Spag6.eSep07	14.4	8.1	
		Spag6.fSep07	11.1	7.5	Stomach, visual cortex
		Spag6.gSep07	8.5	8.3	Cerebellum, cortex
		Spag6.hSep07	9.6	8.5	Oviduct, lung
Mus musculus	*Spag16*	Spag16.aSep07	71.5	6.2	Testis, brain, thymus, testicle, kidney, spleen, liver, heart, lung, ovary, spermatocytes,spinal cord
		Spag16.bSep07	47.0	6.1	Testis, brain, thymus, testicle, kidney, spleen, liver, heart, lung, ovary, spermatocytes,spinal cord
		Spag16.cSep07	45.9	7.1	Testis, embryo, spinal cord, hypothalamus, oocyte, other tissues
		Spag16.dSep07	36.4	6.3	
		Spag16.eSep07	34.1	6.4	Testis, hypothalamus, testicles
		Spag16.fSep07	27.0	7.0	In vitro fertilized eggs
		Spag16.gSep07	21.0	5.1	Testis
Mus musculus	*Spag17*	Spag17.aSep07	249.3	6.4	Testis, lung, cortex, cerebellum, rathke's pouches, testicles, 18-day leptotene and zygotene spermatocytes
		Spag17.bSep07	112.9	6.3	Testis, 18-day leptotene and zygotene spermatocytes
		Spag17.cSep07	33.5	5.6	Testis, 18-day preleptotene spermatocytes, round spermatids
		Spag17.dSep07	23.5	5.3	Testis, head, lung, thymus
		Spag17.eSep07	18.8	7.7	Cerebellum
		Spag17.fSep07-unspliced	16.4	8.6	Cerebellum
		Spag17.gSep07	15.0	7.3	Testicles

Data were extracted from the NCBI AceView database.

Table IV

Amino Acid Sequence Identity and Similarity of CPC Proteins in Mice and *Chlamydomonas* (Mouse/*Chlamydomonas*)

Protein[a]	**Theoretical Molecular weight (kDa)**[a]	**Theoretical Isoelectric Point**[a]	**Identity**	**Similarity**
SPEF2/CPC1	199/205	5.62/5.3	24%	34%
HYDIN	582/488	6.33/6.01	29%	44%
Pcdp1/FAP221	97/104	8.66/6.75	24%	34%
CFAP54/FAP54	353/317	8.37/7.79	23%	10%
SPAG6/PF16	55/60	6.76/5.94	65%	81%
SPAG16/PF20	71/66	6.01/7.86	35%	52%
SPAG17/PF6	263/237	6.05/4.84	20%	28%

[a] Mouse/Chlamydomonas.

Alignments were performed for the full length orthologues, isoforms with MacVector Software, V 12.7.5. The percentage of identity and similarity is based on the entire protein sequence alignment.