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Review
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Review

Formation and function of sperm tail structures in association with sperm motility defects†

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Abstract

Male infertility is an increasing problem partly due to inherited genetic variations. Mutations in genes involved in formation of the sperm tail cause motility defects and thus male infertility. Therefore, it is crucial to understand the protein networks required for sperm differentiation. Sperm motility is produced through activation of the sperm flagellum, which core structure, the axoneme, resembles motile cilia. In addition to this, cytoskeletal axonemal structure sperm tail motility requires various accessory structures. These structures are important for the integrity of the long tail, sperm capacitation, and generation of energy during sperm passage to fertilize the oocyte. This review discusses the current knowledge of mechanisms required for formation of the sperm tail structures and their effect on fertility. The recent research based on animal models and genetic variants in relation to sperm tail formation and function provides insights into the events leading to fertile sperm production. Here we compile a view of proteins involved in sperm tail development and summarize the current knowledge of factors contributing to reduced sperm motility, asthenozoospermia, underline the mechanisms which require further research, and discuss related clinical aspects on human male infertility.

Summary Sentence

This review studies the known factors contributing to male fertility through production of sperm motility by the sperm tail.

Key words: sperm, motility, tail, ODF, fibrous sheath, axoneme, mitochondrial sheath, connecting piece, male fertility.

Introduction

Male infertility is often caused by impaired sperm motility. Thus, understanding the complex process of sperm tail formation and function is essential for solving male infertility issues. As a last phase of spermatogenesis haploid round spermatids differentiate during spermiogenesis, a process where the nucleus is condensed, the acrosome and sperm tail are formed, and excess cytoplasm is discarded. Animal models are widely used to understand male fertility since sperm tail development in vitro is still not established. Mouse and human spermatogenesis are conserved, and spermiogenic steps and cell types are similar; thus, knockout (KO) mouse models for specific spermatogenesis-related genes are valuable tools for understanding protein functions and networks required for male fertility (Table 1). Spermiogenesis in mice can be divided into 16 steps. At steps 1–8, the nucleus appears round, the acrosome flattens, and the axoneme begins to elongate from the distal centriole. Since the sperm tail is a specialized form of the cilium, similar molecular mechanisms are required for cilia and sperm tail formation. Motile cilia and flagella (sperm tail) contain a 9 + 2 microtubule structure. During steps 9–14 in the mouse, a transient microtubular platform, the manchette, surrounds the distal part of the sperm head participating in shaping the head and delivering the proteins to the developing tail. Recently, the importance of correct protein transport during spermatid

Table 1. KO mouse models affecting the formation of sperm tail accessory structure. Axonemal defects have been recently reviewed in [17].

Gene	Spermiogenesis phenotype	Identified interactions	Other identified or suggested roles	Reference
Akap4	Fibrous sheat dysplasia	AKAP3, FSIP1, FSIP2		[56,57]
Azi1/Cep131	Short tail, disorganized sperm tail structures, ectopic and elongated manchette	BBS4	Conserved, but nonessential trafficking role in ciliogenesis, localizes to centriolar satellites and the transition zone, and traffics along microtubules	[36]
Centrobin	Ectopic and asymmetric perinuclear ring and manchette, detached centrosome, decapitated and disorganized tails	KERATIN 5, TUBULIN	Required for centriole duplication and cytokinesis	[115]
CFAP157	Axonemal loop, lack of FS and clustered mitochondria	CEP350, TUBULIN	Localized to basal bodies	[38]
E-Map-115	Ectopic manchette along regions of the nucleus that normally do not display manchette, tail appears normal	KINESIN 1	Stabilizing and reorganizing microtubules	[116]
Fused	Periaxonemal abnormalities, manchette elongated and malformed, acroplaxome affected	KIF27, ODF1	Constructing or maintaining the central pair apparatus of the vertebrate 9+2 axoneme in multi-ciliated tissues	[117]
Gopc	Lack of the acrosome, lack of postacrosomal sheath and the posterior ring, misplaced perinuclear ring, ectopic and misplaced manchette. Impaired mitochondria sheath assembly in the epididymal spermatozoa, coiled flagella	GOLGIN-160, RAB6A, GRID2, BECN1, RHOQ, ACCN3, CFTR, CSPG5	Trafficking of a subset of plasma membrane proteins	[90,118]
Hook1	Manchette elongated, knobbed-like shape of the head, possibly weak head tail connection, bending of the tail	RIM-BP3	Link endocytic membrane trafficking to the microtubule cytoskeleton	[119]
Ift88	No axoneme, disorganized tail components, malformed HTCA, ectopic perinuclear ring, manchette elongated	GMAP210	Part of IFT complex B	[3]
Iqcg	Short tail and disorganized sperm tail structures, elongated manchette	CALMODULIN	Not known, expressed in motile cilia	[120]
Katanin P80	Sperm tail motility affected, manchette elongated, knobbed-like head	KATANIN60	MT severing	[121]
Kif3A	No axoneme, disorganized tail components, manchette elongated, knobbed-like shape of the head	KIF3B, KAP, MNS1, KBP	IFT anterograde motor	[12]
Klc3	Unevenly distributed and malformed mitochondria, abnormal motility	VDAC2, LRGUK1	Involved in mitochondria transport	[87,122]
Ksr2	Detached tails, connecting piece disrupted, disorganized accessory structures, altered motility	Interacts with a number of signaling components of the RAS/MAPK pathway and kinases and phosphatases	Scaffold protein for the Raf/MEK/ERK/MAPK signaling pathway	[123]
Lrguk-1	Short tail, acrosome and acroplaxome detached, manchette MTs unevenly distributed, elongated manchette	HOOK2	Role in MT organization	[124]
Meig1	Disorganized sperm tail structures, disrupted manchette structure reported, round or detached heads	PACRG, SPAG16	Regulation of meiosis	[23,25,26]
Mns1	Short tail, disorganized sperm tail structures, immotility	MFN2	*Mns1* KO also show situs inversus and hydrocephalus	[125]
Odf1	Detached head, abnormal ODF and MS, decreased motility	ODF2, SPAG4, KLC3, OIP1, SPAG5	ODF and connecting piece formation	[43]
Pacrg	Disorganized sperm tail structures, disrupted manchette structure, round or detached heads	MEIG1	In *Chlamydomonas reinhardtii*, PACRG has been shown to be a component of the centriole/basal bodies [126], whereas in *Trypanosoma brucei* PACRG stabilize the outer doublet MTs of the axoneme [127].	[25]

Table 1. Continued

Gene	Spermiogenesis phenotype	Identified interactions	Other identified or suggested roles	Reference
Rabl2	Sperm structure appeared superficially normal, motility affected, 17% shorter tails	IFT27, IFT81, IFT172, Cargo proteins: ATP6V1E1, EB1, HK1, HSP4AL, LDHC	Protein delivery to the flagellum	[128]
Ropn1	Moderately impaired motility	AKAP3	FS formation	[62]
Ropn1l	Slightly decreased motility	AKAP3	FS formation	[62]
Sepp1	Truncated MS, extruded dynein doublets, annulus detached from mid piece	APOER2	Selenium transport	[89]
Sept12	Disorganized sperm annulus, bent tail, reduced motility, nuclear damage	NDC1, SEPT6, SEPT11, α- and β-tubulins	Annulus formation	[72,75,129–132]
Sept2	disorganized sperm annulus, bent tail, reduced motility and loss of the SEPT ring structure at the sperm annulus	Forms a filamentous structure with SEPT7, SEPT 6, SEPT2, SEPT4	Annulus formation	[75]
Sept4	No annulus, bent tail, mitochondria variable size, irregular appearance, reduced membrane material, retention of droplet in neck area	DNAJB13	Annulus formation	[74,81,133]
Slc22a14	Annulus disorganization, motility defect			[77]
Spag16	Decreased motility, double mutant (Spag16l and s) causes axonemal and ODF defects	MEIG1	Ortholog of the *Chlamydomonas PF20* gene, which localized to the axonemal central apparatus regulating flagellar motility [134]	[23]
Spata6	Detached heads, connecting piece disrupted, misplaced annulus and mitochondria, incomplete ODF and axoneme	MYL6, MYH10, MYH11, MYH14	Involved in myosin-based microfilament transport	[37]
Spef2	Short tail, disorganized sperm tail structures immotility	IFT20	*Spef2* KO affects the motile cilia motion	[30,135]
Spem1	Head bend back, mid piece wrapped around head, retained cytoplasm	RANBP17, UBQLN1	Nucleocytoplasmic transport	[136,137]
Tat1	Incomplete oval annulus, abnormal MS, bent tail, immotility	CFTR	Regulation of Cl(-) and HCO(3)(-) fluxes during sperm capacitation	[76]
Tekt4	Decreased motility		-	[138]
Tssk4	Disorganized annulus, some axonemal doublet MTs absent, bent tail, decreased motility	ODF2	Annulus formation, maintenance of sperm tail integrity	[139]
Ube2b	Mislocation of the longitudinal columns of the FS, head shape, and MS abnormalities, acrosomal defects, ectopic manchette	RAD18	Ubiquitin pathway and protein degradation	[140,141]

differentiation has been recognized. Intramanchette transport (IMT) has been suggested to resemble intraflagellar transport (IFT) due to the identified importance of IFT proteins for protein transport through the manchette [1]. IMT has been demonstrated to store and deliver structural sperm tail proteins to the basal body region [2,3]. Sperm tail accessory structures develop after the axoneme has been formed. The fibrous sheath (FS) starts to structure along the principal piece from tip to base orientation, and outer dense fibers (ODFs) develop to surround the axoneme in the principal and mid piece. During the last steps of spermiogenesis, mitochondria are assembled helically around the ODFs in the mid piece of the sperm tail. While dynein arms in the axoneme provide the motor force for sperm tail motility, all accessory structures are required for efficient fertilization capacity of sperm. They stabilize the long axoneme, and provide support for the sperm tail movement and metabolic pathways for energy production. Specific roles of each sperm tail structure are summarized in this review.

Recently, proteomic studies of human sperm have identified more than 1000 proteins associated with the sperm tail structures [4]. These data highlight the complexity of sperm tail and the possibilities of causative genes for asthenozoospermia. A large proportion of identified proteins (26%) were related to metabolism and energy production, lipid metabolism in particular. The occurrence of such proteins suggests that fatty acids are an energy substrate for sperm motility and the presence of peroxisomal pathways in sperm [4]. Identification of proteins involved in formation and transport of sperm components adds to the evidence that defects in these systems contribute to male infertility.

Sperm tail formation

The ultrastructure of the mammalian flagellum is highly conserved and is structurally divided into four major parts: the connecting piece, the mid piece, the principal piece, and the end piece. The axoneme extends from the remnant of the centriole attached to the implantation fossa of the nucleus and is the core structure along the length of the sperm tail (Figure 1). The axoneme is surrounded by the accessory structures ODF, FS, and the mitochondrial sheath (MS)

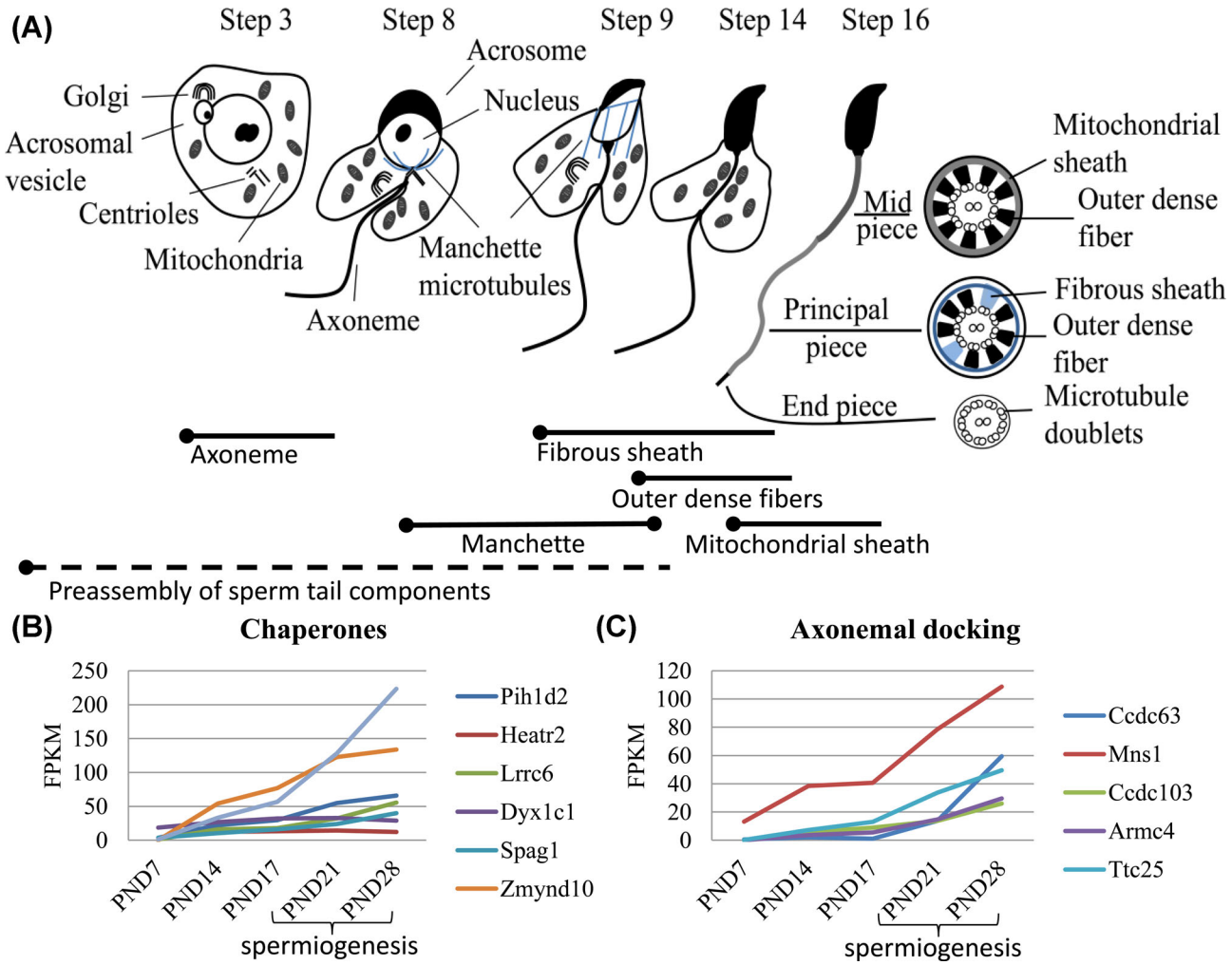

Figure 1. Overview of mouse spermiogenesis and expression of dynein arm preassembly genes. (A) The axoneme elongation begins at early spermiogenesis originating from the distal centriole (step 3). The transient manchette microtubules are assembled during steps 8–9, and the sperm tail axoneme protrudes outside the cytoplasm. In mature sperm tail (step 16), the manchette has disappeared and the accessory structures have been organized around the axoneme. Intra manchette transport is required for protein transport to the basal body region. Outer dense fibers are found in the mid and principal piece, fibrous sheath in the principal piece, and mitochondrial sheath is formed behind the migrating annulus along the mid piece (step 14). The approximate timing of formation of sperm tail structures is indicated with solid line and possible timing of preassembly of sperm tail components with dashed line. (B and C) Gene expression patterns of known dynein arm preassembly genes during the first wave of spermatogenesis. Genes required for dynein arm preassembly in motile cilia are highly expressed during spermiogenesis indicating an important role in sperm tail formation as well as in motile cilia. PND = post natal day, FPKM = fragments per kilobase million.

(Figure 1). The connecting piece contains the basal body and connects the head and tail together. The mid piece contains all mitochondria found in the sperm and nine ODFs surrounding the axoneme. In the principal piece of the sperm tail, two ODFs are replaced by the longitudinal columns of the FS, which are connected to each other by transverse ribs (TR). The end piece of the sperm tail contains only the axoneme surrounded by the plasma membrane.

Factors contributing to sperm tail formation can be divided into three categories: (1) preassembly and transport of sperm tail components, (2) structural assembly of the axoneme, and (3) structural assembly of the accessory structures. Each of these categories is discussed in this review and summarized in Figure 2.

Preassembly and transport of sperm tail components

Protein preassembly prior to transport to the sperm tail

The correct protein modification and preassembly of structural components prior to transport to the developing tail may play an important role in sperm tail motility. It has been shown that dynein arms are preassembled in the cytoplasm prior to transport to motile cilia [5–7]. Dynein axonemal assembly factor 2 (DNAAF2), leucine-rich repeat-containing 6 (LRRC6), and PIH domain containing 3 (PIH1D3) have been reported to be essential for cytoplasmic assembly of the outer dynein arms (ODA) and inner dynein arms (IDA) [8–10]. Mutations in these genes cause the absence of ODA and IDA, causing defective axoneme formation. It was also recently shown that the mutations in dynein arm preassembly genes *Lrrc6* and *Zmyd10* (zinc finger, MYND-type containing 10) affect dynein arm assembly in spermatids, but the role of most assembly factors is unknown. A mutation in *Mns1* (Meiosis Specific Nuclear Structural 1), which results in lack of dynein arms in motile cilia, disrupts the whole sperm tail structure. Therefore, the exact role of these preassembly genes should be studied during spermiogenesis in order to establish the effect on male fertility. Our studies of gene expression patterns during the first wave of mouse spermatogenesis show that the dynein

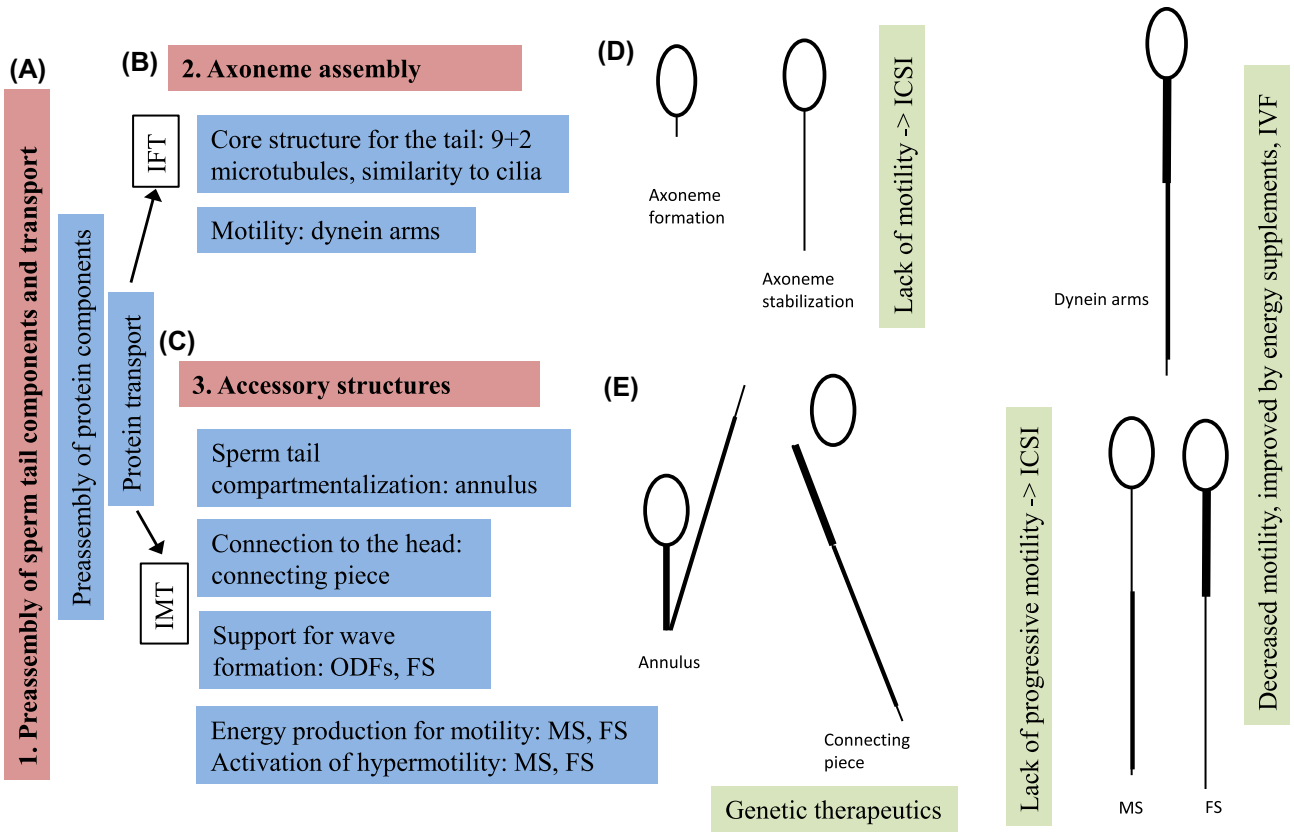

Figure 2. The factors contributing to sperm tail formation and motility and overview of defects. (A) Protein preassembly and transport to the assembly site are required for correct axoneme and accessory structure formation (1). Intraflagellar transport (IFT) functions in axoneme assembly and possibly at later steps in transport of accessory structure proteins. However, the exact transport pathways in later steps of spermiogenesis are not known. Intramanchette transport (IMT) is required for transport of accessory structure proteins through the manchette to the basal body region. (B) The axoneme is the core structure of the sperm tail (2) and contains dynein arms, which function as motors for the motility. The axonemal structure is comparable in motile cilia and sperm tail and mutations in axonemal genes cause male infertility and PCD, although differences exist. (C) All accessory structures are required for correct sperm tail motility (3). Specific functions of accessory structures are presented for the fibrous sheath (FS), outer dense fibers (ODFs), annulus, connecting piece, and mitochondrial sheath (MS). (D) Overview of axonemal defects and the affected structures, e.g. lack of protein transport to the axoneme results in missing sperm tail and problems in assembly of the central pair microtubules cause instability of the axoneme and short sperm tails. Lack of dynein arms affects motility with normal length of the tail. (E) Overview of accessory structure defects and the affected structures. Depletion of proteins involved in specific structures causes distinguishable phenotypes, which can be used for identification of causative genes and evaluation of the severity of infertility and treatment. Annulus and connecting piece defects often lead to total lack of progressive motility. Problems in MS of FS formation reduce sperm motility, which may be improved by alternative metabolic pathways. To overcome the infertility caused by the motility defect, intracytoplasmic sperm injection (ICSI) or energy supplement and in vitro fertilization (IVF) may be utilized. The possible therapeutics is indicated in green boxes.

arm preassembly genes are indeed expressed during spermatogenesis with increasing expression pattern (Figure 1B and C). Thus, it can be expected that at least preassembly of dynein arms is also required during sperm tail formation and components of accessory structures may require similar mechanisms. The protein modification and preassembly processes have been poorly studied, but should be addressed in the future.

Assembly of sperm tail structures requires protein transport to the assembly sites

It has been demonstrated that IFT is the mechanism that transports proteins and protein complexes to the developing cilia and is also required for sperm tail axoneme formation [11,12]. It appears that the axoneme is formed using IFT, and thereafter the manchette is formed for IMT toward the developing tail. The timing of the manchette suggests that it has a role in transport of sperm tail accessory structure proteins. Indeed, it has been shown that many sperm tail components are transiently localized in the manchette [1]. IFT and IMT appear to have a common transport mechanism since IFT motor proteins and transport complex proteins are present in the manchette. The current knowledge of proteins involved in IMT and their cargo was recently reviewed [1]. However, in addition to the IFT and IMT, it is probable that other transport mechanisms exist. The ODFs and FS are composed in opposite directions indicating different transport systems. The annulus migrates toward the mid piece FS border in late spermiogenesis and the mitochondria are assembled as a helical structure behind the annulus. All these processes are dependent on well co-ordinated protein localization to the assembly site. Based on the current knowledge, there are important factors such as Kinesin Family Member 3A (KIF3A), Intraflagellar transport proteins 88 (IFT88), and 20 (IFT20), which are known to be involved in protein transport and their depletion affects sperm tail formation. However, little is known about the specific motor/linker/cargo complexes during spermiogenesis and this is a field that requires extensive future studies.

For male fertility, counseling the important factor would be identification of testis-specific cargo binding proteins. The widely expressed proteins required for IFT usually cause embryonic lethal phenotypes, where the fertility is not an issue. However, in patients with milder phenotypes even with multiorgan defects, it would be beneficial to be able to predict the fertility status later in life. The correlation between gene function in ciliated cells and sperm development is poorly known, but clear differences seem to exist. Furthermore, the IMT is spermatogenesis specific; thus, gene functions and protein delivery through the manchette may well present spermatid-specific roles for known or novel IFT/IMT-related genes. Thus, identification of transport complexes in protein trafficking to the developing sperm tail is of great importance.

Components and role of the axoneme

Sperm tail axoneme formation resembles motile cilia assembly with testis-specific isoforms

Sperm axoneme formation starts during early spermiogenesis. In round spermatids, the axonemal structures first start to elongate in the cytoplasm making contact with the nucleus before protruding outside the cytoplasm (Figure 1). The axoneme is a microtubule-based structure of nine outer doublet microtubules and central doublets (9 + 2) associated with radial spokes and dynein arms (Figure 3B). Dynein arms within the axoneme provide the motor apparatus for the movement of the sperm tail [13]. The exact structure of the axoneme and dynein-based motility have been recently reviewed by [14]. Because the sperm tail axoneme resembles motile cilia, male infertility due to malformations of the axonemal structure is often associated with primary ciliary dyskinesia (PCD). However, male infertility is not systematically investigated and often not recorded in cases of PCD. Recent reviews have considered causative gene mutations for PCD [15] and their association with sperm motility [16] and male infertility [17]. Mutations in more than 30 genes have been identified in cases of PCD including dynein arm preassembly genes. Furthermore, defects in the axonemal ODA genes dynein axonemal heavy chain 5 (DNAH5) and dynein axonemal intermediate chain 1 (DNAI1) cause reduced sperm motility, although sperm axoneme structure appears intact [18,19]. Mutations in IDA-related coiled-coil domain-containing proteins 39 (CCDC39) and 40 (CCDC40) cause reduced sperm motility and absence of IDA [20].

Defects in central pair-related genes have also been shown to cause male infertility. In humans, depletion of Hydin causes PCD, and spermatozoa appear rigid and completely immotile [21]. Several sperm-associated antigen (Spag) genes (*Spag6, Spag16*, and *Spag17*) have been shown to be important for central pair complex function. Total loss of *Spag6* causes infertility due to missing axonemal central pair and disorganized ODFs [22]. However, depletion of the SPAG16L isoform causes only sperm motility defects with intact axonemal structure [23]. Another isoform, SPAG16S, has been shown to interact with meiosis expressed gene 1 (MEIG1) [24] and the localization of SPAG16L in the manchette is dependent on MEIG1 [25]. Depletion of MEIG1 disrupts all sperm tail structures [26], and it may function in protein transport through the manchette. Although the SPAG16 resembles the Chlamydomonas PF20 protein, which is a linker between the central microtubules, it appears to have additional roles during mammalian spermatogenesis. It is not evident if SPAG16 is only transported through the manchette to serve as a structural protein in the sperm tail or if it has a role in protein transport [25]. The sperm tail-specific SPAG16S may compensate for the loss of the ciliary variant during spermiogenesis or it may have an independent role. SPAG17 is also localized to the central pair of the sperm tail axoneme [27] and forms an interactome with SPAG6S and SPAG16L [27,28]. It can be concluded that the CP proteins are crucial for male fertility, but may have sperm-specific isoforms and functions.

Differences between cilia and flagella formation clearly do exist, since some of the PCD genes appear to be more crucial for the sperm tail formation than for ciliary function. These genes include Dynein Axonemal Heavy Chain 1 (Dnah1) and Sperm flagellar protein 2 (Spef2), depletion of which disrupts the formation of the central pair microtubules in the sperm tail, but the ultrastructures of other cilia appear unaffected. However, the function of nasal cilia was not studied in presence of a *Dnah1* mutation [29]. In the *Spef2* KO mouse, beat frequency of the tracheal cilia is affected [30]. Thus, the effect of certain axonemal genes appears to be more pronounced in the sperm tail. At the same time, some of the PCD mutations do not seem to affect sperm tail formation. This may be due to a testis-specific homolog, which may compensate for the lack of a protein product during spermatogenesis as was demonstrated for an outer dynein arm docking complex Coiled-Coil Domain Containing 114 (CCDC114), which has a testis-specific homolog CCDC63 [31]. Differences in gene isoforms between tissues result in variable or tissue-specific phenotypes depending on the site of the mutation. The testis-specific isoforms of cilia genes may also serve different functions during spermatid elongation and in mature sperm. Overall, recent reports allude to differences between cilia and flagella formation, but the exact mechanisms require extensive additional studies, which is a necessity in developing fertility counseling for PCD patients.

Components and role of the accessory structures

The connecting piece is required for intact head/tail connection and motility

The pair of centrioles in the cytoplasm of round spermatids forms the basal body and surrounding connecting piece, which anchors the elongating sperm tail to the posterior pole of the nucleus (Figure 1). After attachment to the sperm nuclear envelope, the centrioles are enclosed by nine longitudinal segmented columns and the capitulum. The capitulum links the connecting piece to the sperm head by association with the implantation fossa at the nuclear surface (Figure 3A) [32]. The segmented columns are attached to the ODFs at their caudal ends providing rigid support for the sperm tail motility. It has been speculated that the sperm tail motility is also partly regulated by the connecting piece [33,34].

The correct function of centrosomal proteins is required for basal body and connecting piece formation as demonstrated by depletion of the centriolar protein Centrin 1. Centrin 1 appears to have a role in basal body attachment to the nuclear membrane. The lack of Centrin 1 and basal body attachment prevent the nuclear implantation fossa formation and result in degradation of the basal body complex [35]. In addition, the protein transport mechanisms appear to be important for connecting piece formation as well as for other sperm tail structures. Recent studies have indicated that centrosomal protein 131 kDa (CEP131) is involved in microtubule-based trafficking, but is dispensable in other ciliated cells except the sperm flagella [36]. Depletion of the *Cep131* causes misalignment of the centrioles in the implantation fossa and short and disorganized sperm tails [36]. The malformed head shape and manchette support the hypothesis that CEP131 has a role in microtubule-based trafficking, but CEP131 has not been localized in the manchette or sperm tail; thus, it cannot be concluded that it has a role in IMT or IFT based on the published

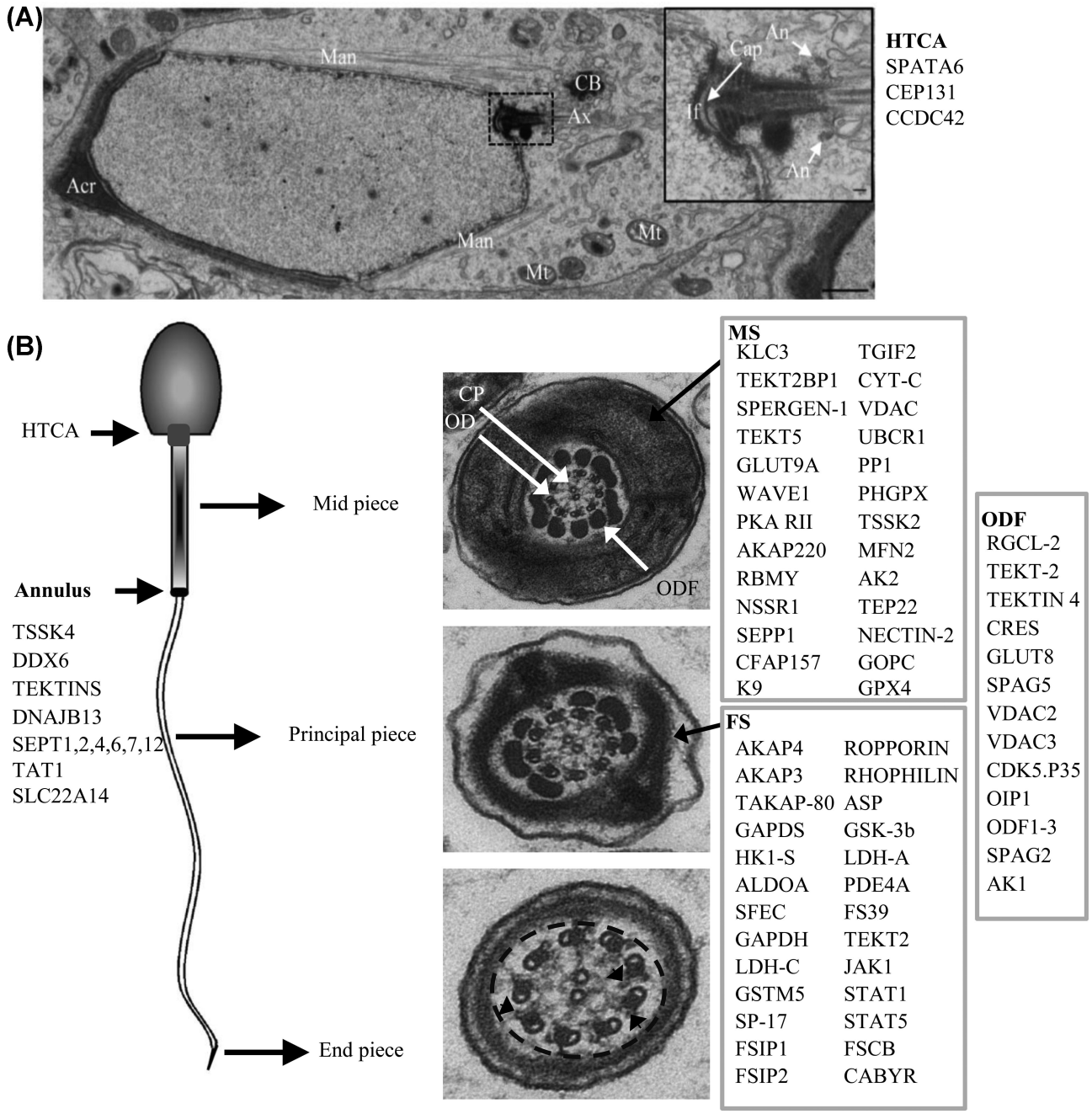

Figure 3. Structures of the sperm tail and associated proteins. (A) The connecting piece attaches the sperm tail to the head (step 11 spermatid). The centrioles are attached to the nuclear membrane by the implantation fossa and capitulum. Surrounding nine longitudinal segmented columns link the head tail coupling apparatus (HTCA) to the outer dense fibers (ODFs). The manchette (Man) is a transient skirt-like microtubular structure transporting proteins to the developing sperm tail. Acr: acrosome, CB: chromatoid body, Mt: mitochondria, Ax: axoneme, If: implantation fossa, Cap: capitulum, An: annulus. (B) Mature sperm tail structures and associated proteins. Schematic presentation of the spermatozoa including the HTCA, mid piece, annulus, principal, and end piece of the sperm tail. The cross section of the mid piece contains the axoneme (CP = central pair, OD = outer doublet microtubules), ODF, and mitochondrial sheath (MS). The cross section of the principal piece includes the axoneme, ODFs, and fibrous sheath (FS). The axonemal structure is retained in the end piece of the sperm tail (dashed line). Proteins associated with the annulus, MS, ODFs, and FS are indicated with arrows and dynein arms with arrowheads.

results [36]. The localization of CEP131 in the basal body region implicates a possible role in attachment of the connecting piece to the head and possibly a role in protein transition to the tail since it has been also localized to the transition zone in cilia [36]. However, another protein required for formation of segmented columns and capitulum, the spermatogenesis-specific protein SPATA6, interacts with myosin and is localized in the manchette, where it may be involved in protein delivery to the developing connecting piece [37]. Recently, a novel interacting partner of a centrosomal protein CEP350, Cilia And Flagella Associated Protein 157 (CFAP157), was identified to cause sperm axonemal loop, absence of FS, and clustered mitochondria [38]. Although CFAP157 is expressed in all

tissues with motile cilia, the depletion of the protein only affects sperm formation. Additionally, coiled-coil domain containing gene 42 (CCDC42) has been shown to have a role in formation of the head tail coupling apparatus (HTCA) and is crucial for the initiation of sperm axoneme assembly, but is not required for ciliogenesis [39].

During spermiogenesis, the distal centriole diminishes, but the proximal centriole is delivered to the oocyte. Proximal centriole serves as the sperm aster that assembles the direct pronuclear migration and fusion, and the connecting piece serves as a detachment site for the sperm head during fertilization [40]. Furthermore, proteasomes have been localized in the connecting piece and protease inhibition has been shown to affect the sperm aster formation [41]. Malfunction of proteins involved in connecting piece formation or function often cause head and tail disconnection, acephalic sperm, leading to male infertility [42]. Thus, a defect caused by malfunction of a connecting piece protein can be expected to cause this profound phenotype with the head/tail detachment being easy to diagnose from a sperm sample. Although the intracytoplasmic sperm injection (ICSI) treatment could be a solution for patients also with connecting piece defects, the possible importance of the sperm centrosome for developing embryo may cause birth defects as an outcome. Therefore, the effect of ICSI with detached head should be investigated prior to recommendation for patients.

Outer dense fibers provide rigidity and contribute to sperm hypermotility

The axoneme is the basis for correct assembly of the sperm tail-specific accessory structures. During sperm tail formation, the axoneme and ODFs elongate in a proximal to distal direction. It has been shown that ODF proteins are transported through the manchette during spermiogenesis. In IFT88 mutant mice, ODF proteins accumulate in the manchette indicating that IMT is required for their transport [3], but the protein complexes involved have not been established. The ODFs consist of keratin-like intermediate filament proteins with a possible role in providing elasticity and structural integrity to the sperm tail. ODFs are attached to the connecting piece and axoneme supporting the stability of these structures. Several components of the ODFs have been identified (Figure 3B). The main structural and only sperm-specific protein is ODF1. Depletion of ODF1 affects the formation of the ODFs, MS, and connecting piece (implantation fossa and segmented columns) leading to detached sperm heads and male infertility [43,44]. Strong connection between the axonemal doublet microtubules and their associated ODFs has been demonstrated [34], but the exact interactions are not known. ODFs also attach the axoneme to the connecting piece, which may be at least partly carried out through interactions between ODF1/SPAG5/SPAG4/SEPT12 [45–48]. The ODF1 interaction with ODF2 most likely accounts for the rigidity of ODFs, since thinning of the ODF layer and missing ODFs was detected in chimeric gene trap *Odf2* mouse model [49]. The functional interactions of ODFs with axonemal doublets along the FS and with the connecting piece are irreplaceable for sperm motility. Furthermore, ODFs detach from the mid piece during capacitation and failure to detach leads to stiff sperm tail mid piece and poor motility [34].

Recent studies have also indicated a more active role for ODFs in sperm motility during capacitation. One isoform of adenylate kinases (AK), AK1, has been localized to ODFs. During high energy consumption, ADP can be used for energy production through catalyzation by AK. Fertilization promoting peptides are essential for sperm capacitation and acrosome reaction and the associated receptor t-complex protein 11 is known to interact with ODF1 [50]. An increase in protein tyrosine phosphorylation of sperm flagellar proteins is also critical for sperm capacitation. In addition to FS, MS, and axonemal proteins, tyrosine phosphorylation of ODF1 [51] and ODF2 [52] has been linked to sperm motility. Recent studies have shown that ODF2 may recruit TSSK4, a member of the testis-specific serine/threonine protein kinase (TSSK) family, to ODF, and TSSK4 may change the modification state of ODF2 to regulate the sperm motility and structure [53]. Furthermore, a fluorescent thiol-selective labeling agent, monobromobimane, specifically binds to the N-terminal domain cysteine of ODF1 during rat spermatogenesis [54]. During maturation in the epididymis, mammalian sperm proteins undergo thiol group oxidation to form disulfides bonds, which are involved in chromatin condensation and tail organelle stabilization. Thus, the capacitation and hyperactivation of sperm motility in the female reproductive tract are dependent on the correct function of ODFs. Any mutations in genes involved in these pathways most likely cause infertility due to inability of sperm to reach the oocyte [34].

The sperm tail fibrous sheath includes important pathways for sperm motility

Another supporting structure of the sperm tail is the FS. The important structural role of FS is emphasized by the dysplasia of fibrous sheath (DFS) phenotype, where axonemal and ODF structures are affected by mutations in FS genes. In addition to a structural role, FS has an important role in providing energy for the sperm tail motility. The structure, development, and function of the FS have been reviewed in [55]. Fibrous sheath is composed of two longitudinal columns, which are attached to ODFs 3 and 8 in an anterior part of the principal piece. In the middle and posterior part of the sperm tail, longitudinal columns of FS replace ODFs and associate with outer microtubular doublets. Longitudinal columns decrease in size toward the end piece and are connected to each other by TR along the whole length of the principal piece. Formation of the FS continues during most of the spermiogenesis elongation phase, and formation of the longitudinal columns and TR occurs in a distal-to-proximal direction. Thus, all proteins required for FS formation need to be transported to the assembly site. More recent studies have identified several members of motility and also metabolism-related pathways in the FS indicating a role in signaling cascades important for sperm fertility. To date, more than 20 FS-related proteins have been documented (Figure 3B). However, two structural main components of the FS are A-kinase anchoring proteins (AKAPs) AKAP3 and AKAP4.

AKAP3 initiates the FS formation and incorporates AKAP4 into the developing FS during later steps [56]. The depletion of AKAP4 results in male infertility due to inability to complete the FS although the ODFs and axoneme appear intact [57]. AKAP3 and glyceraldehyde 3-phosphate dehydrogenase-S (GAPDS) levels were also low in *Akap4* mutant mice, suggesting reduced glycolysis [57] and energy production leading to poor sperm motility [58]. Depletion of GAPDS in mice led to male infertility due to reduced sperm motility with no detectable progressive movement, which was caused by dropped ATP production by almost 90%. Interestingly, mitochondrial oxidative phosphorylation was retained, suggesting that glycolysis is the main energy source for sperm motility [59]. Energy production for sperm motility utilizing glycolysis is incorporated in the FS, which has been proven by localization of most glycolytic enzymes to the FS. Thus, the functional FS is crucial for sperm motility and genetic defects in associated genes are likely to affect the energy production

for movement. This pathway seems to be linked to human male infertility as well since an intronic mutation in *Gapds* and a deletion in *Akap3* and *Akap4* have been reported in DFS patients [60,61]. Interestingly, the Rho signaling pathway through the rhophilin associated protein 1 (ROPN1) and rhophilin are localized to the FS, and integration of AKAP3 to FS was blocked in ROPN1 and ropporin-like gene (ROPN1L) double mutant mice [62]. Decreased motility was also seen in single mutants indicating that Rho signaling is required for sperm motility, although these proteins appear to compensate for each other in FS formation.

The FS has been shown to have an important role in calcium signaling, on the basis that it contains cation channel of sperm (CatSper) ion channel proteins [63] and calcium-binding tyrosine phosphorylation-regulated protein (CABYR), which interacts with a calcium-binding protein Fibrous Sheath CABYR Binding Protein (FSCB) [64]. Calcium signaling controls the hyperactivation of sperm motility in the female reproductive tract and the main ion channel controlling calcium levels is the CatSper complex. Depletion of CatSper subunits causes male infertility due to the inability of sperm to hyperactivate and reach the oocyte [65,66]. CABYR binding to AKAP3 enables the localization of calcium signaling pathway to the FS [67,68]. The sperm-specific lactate dehydrogenase (LDHC) is also localized in the FS and targeted disruption of the gene demonstrated that it is required for capacitation, motility, and fertilizing capacity of sperm [69]. Such observations highlight the importance of FS in energy metabolism, ATP generation for sperm motility, calcium signaling, and as a scaffold for signaling molecules in addition to acting as a structural girdle surrounding the ODFs and axoneme.

Several other proteins, including IFT proteins KIF3A and IFT88 [3,12], have also been localized to the principal piece of the sperm tail, but their role in mature sperm tail has not been established. Localization of Janus Kinase 1 (JAK1) and signal transducer and activator of transcription proteins 1 (STAT1) and 5 (STAT5) in the FS [70] suggests a sperm-specific role for these proteins in addition to their function in the JAK/STAT signaling pathway and activation of the transcription. Further investigations are required to establish the precise function and interactions of various FS proteins. Based on the important role of the FS in energy production, the roles of these proteins are probably related to activation of sperm motility.

The annulus is a diffusion barrier between the sperm tail mid piece and the principal piece

The sperm tail mid piece and FS are separated by the annulus, which is an electron-dense ring structure functioning as a diffusion barrier in mature sperm [71]. The annulus is formed when the axoneme extends outside the cell surface and during late spermiogenesis, prior to MS formation, the annulus moves along the axoneme and ODF structures to the proximal edge of the FS. The mammalian sperm annulus is a complex of septins 1, 2, 4, 6, 7, and 12 [72]. Depletion of septin (SEPT) proteins SEPT4 and SEPT12 is associated with a disorganized annulus, bent sperm tail, and immotility [72–74]. Previous studies also suggest that SEPT12 interacts with α- and β-tubulins [75], and thus may be involved in transport of the annulus along the developing mid piece. Interestingly, the lack of motility in *Sept* mouse models cannot be explained simply by the inability to form the annulus. It has been demonstrated that kinesin motors were unable to move along the sperm tail in absence of SEPT4 and the annulus [73]. It is intriguing to speculate that specific transport mechanisms are required for efficient distribution and consumption of ATP in mature sperm. IFT motor protein KIF3A has been localized to the FS [12], but it seems to be in depletion of most IFT proteins [11]. Thus, it appears that IFT is not the transport mechanism in mature sperm, but other, yet unknown, transport systems are responsible for the capacitation and energy distribution in spermatozoa.

Another annulus protein affecting sperm motility is the testis anion transporter 1 (TAT1) [76]. In both mouse models, *Sept4* and *Tat1*, the mitochondria structure and size were altered in the sperm tail mid piece, but the ATP levels were normal. This result indicates that the problems in MS assembly did not cause the immotility and support the hypothesis that the annulus is required for utilization of ATP along the sperm tail. Furthermore, the phenotype of *Tat1* (*Slc26a8*) null mice corresponds to the depletion of a solute carrier protein SLC22A14, which also appears to have a role in annulus formation and in FS [77]. During epididymal passage, the sperm tails of *Slc22a14* null mice became bent close to the annulus, which is a common feature to defective annulus during capacitation. It has been shown that hypotonic stress, inhibition, or deletion of ion channels induces the hairpin-like bending [78–80].

The *Sept4* null mice have also demonstrated that the annulus functions as a membrane restriction barrier [81]. Basigin, an integral membrane receptor, localizes in the principal piece in spermatozoa isolated from the caput epididymis, and in transition through the epididymis, it translocates to the mid piece of the cauda sperm [82]. However, in *Sept4* null mice, Basigin was present along the whole length of the sperm tail. Basigin is required for placement of monocarboxylate transporters (MCT) in the membrane and MCT2 is colocalized with Basigin in the sperm tail [83]. The role of Basigin and MCT2 in transport of energy substrates through the membrane may contribute to sperm motility. Thus, the annulus seems to function as a barrier for correct localization of membrane receptors during sperm maturation and in mature sperm. In addition, the migration of the annulus is necessary for the transport of the residual body, since it is retained in the neck area instead of the distal mid piece near the annulus in *Sept4* mutant mice. However, the exact causes of the motility defects and importance of the sperm tail compartmentalization require further studies, but it can be expected that various, thus far unknown, proteins affect the sperm motility and therefore male fertility. Even though the protein content of the annulus has been described, the movement and exact physiological functions remain unclear.

Mitochondrial sheath is composed behind the migrating annulus

The KO mouse models of annulus proteins SEPT4 and TAT1 support the hypothesis that the annulus is involved in the organization of the mid piece and MS. However, the presence and correct localization of the annulus is not sufficient for MS formation as was shown by the *Cfap157* null mice [38]. Although the annulus appeared unaffected, the mitochondria were clustered in the neck region. Thus, CFAP157 or other unknown proteins are required for correct organization of the MS. During MS formation, the mitochondria elongate to surround the axoneme and ODFs as end-to-end helices behind the migrating annulus [84,85]. While the functions of sperm mitochondria are similar to those of somatic mitochondria being required for oxidative phosphorylation, several unique proteins or protein isoforms have also been localized to the mid piece (Figure 3B). The relevance of mitochondria in fertile sperm production has been emphasized by several mitochondrial genome mutations [86]. Kinesin light chain 3 (KLC3) binds mitochondria and its inability to bind ODFs leads

to malformations in MS, which results in changes in progressive motility and subfertility [87]. The attachment of mitochondria to ODFs during formation of the mid piece may occur through the interaction between KLC3 and ODF1 [88]. KLC3 is a component of the microtubule molecular motor kinesin 1 and KLC3 interaction with the mitochondrial outer membrane porin protein Voltage Dependent Anion Channel 2 (VDAC2) suggests a role in mediating the movement of mitochondria to the developing mid piece [87]. In addition, a protein required for selenium transport, selenoprotein P (SEPP1), may also be required for correct MS formation [89]. *Sepp1* null and selenium-deficient wild-type mice are infertile due to malformations in the MS, a defect hypothesized to reflect decreased spermatid expression of the selenoprotein phospholipid Glutathione peroxidase 4 (GPX4). GPX4 is the major structural protein of the mitochondrial capsule, which confers stiffness on the outer membrane of the sperm mitochondria. The effect of selenium deficiency also underlines the importance of micronutrients for fertile sperm production. In *Gopc* (Golgi-associated PDZ- and coiled-coil motif-containing protein) null mice, GPX4 was unevenly distributed on the mitochondrial capsule during transport in the epididymis [90]. Thus, GOPC appears to have a role in MS organization in addition to acrosome formation. The adhesion between the individual mitochondria in *Gopc* null MS was maintained by spermatogenesis-associated protein 19 (SPATA19). *Spata19* null mice are infertile and show motility defects due to irregular organization of mitochondria and reduced ATP production [91].

Based on these studies, it can be speculated that GOPC, GPX4, and KLC3 are necessary to maintain the connection between ODFs and mitochondria, while SPATA19 prevents dispersal of mitochondria. The sperm mitochondria-associated cysteine-rich protein (SMCP) has also been suggested to have a stabilizing role on the mitochondrial capsule. *Smcp* null mice are fertile, but sperm tail motility seems to be affected [92]. Mitofusin 2 (MFN2), a protein that participates in regulating mitochondrial associations to subcellular organelles, has been localized to the mid piece of the sperm tail [93]. The exact role of MFN2 has not been established, but it has been implicated as a factor for changes in mitochondrial activity during cryopreservation [94]. Mitochondria appear to be especially sensitive to changes induced by cooling and cryopreservation [95].

Activation of sperm motility by calcium signaling and production of energy

The fertilizing capacity of sperm requires efficient energy production and timely activation of motility. Energy production for sperm tail motility through glycolysis and oxidative phosphorylation (OXPHOS) has recently been reviewed [96]. Early studies of bull sperm motility already underlined the importance of OXPHOS and glycolysis, since the sperm motility is dependent on constant ATP production [97–100]. It has been suggested that sperm can survive solely from ATP from glycolysis, but OXPHOS is required for differentiation and maturation. However, the preferred metabolic pathway for energy production differs between species. Glycolysis has been suggested to act as a spatial ATP buffering system, transferring energy (ATP) synthesized by respiration in the mitochondria located in the basal part of the flagellum to the distal part. Mouse sperm were shown to maintain vigorous motility in the presence of substrates either for glycolysis or OXPHOS. By contrast, inhibition of glycolysis by alpha-chlorohydrine caused a significant decrease in the bend angle of the flagellar bending wave, sliding velocity of outer doublet microtubules and ATP content even in the presence of OXPHOS substrates [101]. Several mouse models have established the importance of glycolysis and OXPHOS for sperm tail motility, although even with compromised motility the male mice are fertile. It appears that different energy metabolic pathways can, at least to some extent, compensate for variations in substrate supply.

In addition to energy production, the hyperactivation of sperm motility in the female reproductive tract is pivotal for fertility. The CatSper channels appear to be the main regulator of the calcium signaling and therefore hypermotility in sperm across species. Sperm encounters complex chemical and physiological barriers on the way to fertilize the oocyte. In order to overcome these obstacles, sperm must sense the environmental cues and change its swimming pattern, which is achieved by ion channels. Recent studies have underlined additional ion channels, which regulate CatSper activity and are important for physiological conditions in mature sperm [65,102]. Specific ion channels in the mid piece have also been identified (e.g. ionotropic purinergic receptor P2×2 [103]), which further emphasizes the importance of sperm tail compartmentalization for fertility. However, the specific ion channels differ between species and thus require further studies to establish their importance and exact roles. Although the morphological changes in sperm structure and motility defects prior to capacitation are easily visible, there are various factors influencing male fertility that can be detected only by specific biomarkers. Identification of these mechanisms enables development of fertility-related diagnostics and therapeutics. It can be hypothesized that addition of different substrates in the environment of the sperm could improve the motility in presence of specific mutations. On the other hand, the specificity of CatSper channels to sperm makes them an intriguing target for development of contraceptives [66,104].

Sperm tail defects in relation to human male infertility

It is well known that sperm tail malformations and motility defects cause male infertility. However, the underlining cause for the defects is often unknown. Human male infertility has been associated with several mutations in sperm tail accessory structures in addition to PCD. Furthermore, although the axonemal structure in cilia and sperm tail flagella is similar, it is becoming evident that differences in formation and function exist [105]. In many PCD cases, male infertility is reported, but the sperm tail phenotype has not been investigated. Mutations in *Dnah1* gene appear to be the most common identified cause for male infertility related to sperm tail formation [106–108]. *Dnah1* has also been associated with PCD [109,110], but based on the current knowledge it is not crucial for dynein arm formation in cilia. DnaJ homolog subfamily B member 13 (DNAJB13) causes depletion of the axonemal central pair in motile cilia [111], but has been transiently localized to the annulus in sperm tail [112]. Mutation in a male patient also resulted in infertility, but the exact effect on sperm tail structure is not known [111]. Furthermore, the importance of nonstructural proteins for sperm tail development has been recently recognized. Although the structural proteins have been studied and many are known, the events prior to structural assembly are poorly understood. Furthermore, mutations affecting the capacitation and hyperactivation of sperm, such as the *CATSPER1* and *CATSPER2* genes coding for CatSper subunits, have been identified in infertile male patients [113,114] and other genes affecting the same pathway are good candidates for infertility screening. In

addition to structural proteins, human male fertility also relies on proteins in preassembly and transport pathways prior to structural assembly and in storage and enzymatic pathways in mature sperm. The heterogeneity of male infertility substantially hampers identification of causal genes, but clear phenotype and recent advancements in sequencing technologies enable also identification of genetic causes of male infertility.

For resolving male factor infertility, it is crucial to identify and understand the factors and mechanisms contributing to fertile sperm. The identified genes affecting sperm motility can be used as biomarkers for male fertility. For example, the known gene mutations causing PCD and sperm tail phenotype can be utilized for prediction of male infertility. The counseling of PCD patients for their fertility status is an important factor, and therefore the effect of identified PCD genes on male fertility should be studied. Thus far, the effect has been poorly reported and therefore the usability of PCD genes as biomarkers also for male fertility is inadequate. Sperm-specific defects can be expected to rise from gene mutations affecting sperm tail accessory structures and motility pathways. However, very limited number of mutations have been identified in human patients [17,114], which indicates that additional studies are needed. However, the expression levels of known genes in sperm tail structures can be used as an indicator of fertility potential and these genes are good candidates for causative mutations. It is crucial to underline the roles of proteins in different tail structures for prediction of impact on fertilizing potential and developing embryo. Although ICSI can be used for fertilization of the oocyte in vitro, the effects of sperm tail malformations on offspring are not well understood. Thus, it would be beneficial to introduce as natural conditions for in vitro fertilization (IVF) as possible. In the case of reduced motility, some energy supplements could be developed to increase motility in vitro. This ensures some level of natural selection for the offspring. Furthermore, the recent development in genome editing methods and RNA therapeutics underline intriguing prospects on genetic correction of inherited mutations. Genome editing involves major ethical issues, which need to be solved prior to therapeutic use. However, RNA therapeutics give promise for more short-term development of male infertility treatments. Research is underway for new genetic therapeutics and diagnostics for genetic diseases and is an exciting field for treatment of male infertility as well.

Conclusion

Sperm tail formation is a unique process, although the axoneme structure and protein transport mechanisms resemble motile cilia. The results from various studies denote the importance of specific proteins for sperm tail basal body, HTCA, and axoneme formation. Due to the specialized long flagellum and required wave form for motility, the axoneme alone is not sufficient to provide the necessary rigidity and energy for sperm in order to reach and fertilize the oocyte. The first phase required for formation of motile sperm after protein expression is the preassembly of required sperm tail components and transport to the assembly site. Thus far, studies have concentrated on the structural composition of the sperm tail structures; however, recent studies have indicated that regulation and transport prior to structural assembly are important factors in producing fertile sperm. These mechanisms are poorly studied and should be addressed in future investigations.

Since the role of the sperm tail is to produce motility, it is reasonable to conclude that mutations affecting any part of the sperm tail result in motility defects including biochemical properties required for capacitation and hypermotility. This hypothesis has been proven by previous studies, but additional investigations are needed for identification of the exact roles of axonemal and accessory structure proteins, differences between cilia and sperm tail, and the role of nonstructural proteins in order to decipher the genetic causes of male infertility. Mutations in genes affecting specific structures of the sperm tail result in common phenotypes (Figure 2, Table 1), which can be used in investigation of the cause of infertility. Although IVF and ICSI can overcome the lack of sperm motility, specific structural defects such as centrosome and axoneme malformation may influence the outcome of these technologies. Thus, the understanding of the effects of genomic mutations and development of genetic therapeutics and diagnostics are of great importance.

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