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Review
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Review

The coupling apparatus of the sperm head and tail†

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Abstract

A strong sperm head–tail coupling apparatus (HTCA) is needed to ensure the integrity of spermatozoa during their fierce competition to fertilize the egg. A lot of HTCA-specific components have evolved to strengthen the attachment of the tail to the implantation fossa at the sperm head. Defects in HTCA formation lead to acephalic spermatozoa syndrome and pathologies of some male infertility. Recent studies have provided insights into the pathogenic molecular mechanisms of acephalic spermatozoa syndrome. Here, we summarize the proteins involved in sperm neck development and focus on their roles in the formation of HTCA. In addition, we discuss the fine structures of the sperm neck in different species from an evolutionary view, highlighting the potential conservative mechanism of HTCA formation.

Summary Sentence

The head–tail coupling apparatus, which strengthens the attachment of the tail to the sperm head, is essential for male fertility.

Key words: acephalic spermatozoa syndrome, evolution, head–tail coupling apparatus, sperm competition.

Introduction

Millions of spermatozoa compete for the limited chance to fertilize the egg, and only one can be the champion. To win the championship with 60–100 million human spermatozoa, a spermatozoon exhibits unique postcopulatory, intersexual selection to increase its successful chance in the fertilization competition [1] and has to swim at extremely high speeds to arrive at the destination first [2, 3]. Faster spermatozoa have a much greater chance to win during sperm competition, and to achieve greater speed, a wide range of sperm features have evolved, such as size, morphology, metabolism, and motile performance [4]. As an exquisitely designed structure [5], the spermatozoon is made up of two main parts, the head and the flagellum. The core of the sperm head is the nucleus, which carries the haploid genetic material of the male. At the anterior region of the sperm, nuclei lay the acrosome, a special membranous organelle that contains a variety of hydrolases that help the sperm to penetrate the egg's coats [6]. The motility of the spermatozoa is reliant on the flagellum. Usually, the longer the flagellum, the faster the spermatozoa moves [7, 8]. The core of the sperm flagellum is the axoneme, which is composed of "9 + 2" microtubules. The axoneme is surrounded by accessory structures: outer dense fibers (ODFs), the fibrous sheath (FS), and the mitochondrial sheath [9, 10]. The sperm head and flagellum are connected by the head–tail coupling apparatus (HTCA) (also termed the connecting piece or sperm neck). As a long slender structure, a strong sperm HTCA is needed to integrate the sperm head and tail together during

high-speed movement. Any defects of HTCA formation might result in the separation of the sperm head from the tail during movement, causing acephalic spermatozoa syndrome that leads to male infertility [11, 12].

The process of HTCA formation during spermiogenesis

Spermiogenesis is a complex and highly ordered biological process in which spermatids undergo acrosome biogenesis, nuclear chromatin condensation, head shaping, flagella formation, and cytoplasmic removal [13].

The HTCA contains proximal centrioles, distal centrioles, and associated dense material, which includes the capitulum (the dense fibrous plate-like structure) and the segmented columns (Figure 1A) [14]. The formation of the HTCA occurs during spermiogenesis. In mammals, during the initial phase of spermiogenesis, the spermatid contains a round-shaped nucleus with Golgi-derived proacrosomal granules. The acrosome vesicle lies in the perinuclear ring, the mitochondria lie in the periphery of the cytoplasm, and the centrioles can be observed perpendicular to each other [15].

In late round spermatids, the pair of centrioles localizes to the caudal nuclear pole and begins to expand the electron dense material, part of which shows striation. The tail, emanated from the distal centriole, is implanted in the nucleus by a basal plate [16].

At the beginning of the elongation phase, the manchette—an array of microtubules—is attached to the perinuclear ring, which sits just below the leading edge of the acrosome. The nucleus then starts to elongate and condense. Dense material surrounding the centrioles gradually becomes a well-organized structure, which can be clearly recognized as capitulum and basal plate; both are linked by bridging fibers or elements. The capitulum links connecting pieces to sperm heads by bonding with basal plates, electron-dense structures that line the outer nuclear membrane at the implantation fossa [17]. The cytoplasmic lobe forms after the acrosome forms and eventually phagocytosed by the sertoli cell as part of the residual body [18]. The annulus is composed of ring-shaped electron dense granular material and is located caudal to the centrioles, encircling the axoneme, and forming the entrance to the periaxonemal compartment.

At the beginning of the maturation phase, chromosomal condensation is almost complete; both the segmented columns and the capitulum are fully developed and constitute a dense shield that lodges and encloses both centrioles. Excess cytoplasm is removed at the end of the maturation phase, resulting in the formation of cytoplasmic droplet. The disassembly of the manchette occurs early in the maturation phase followed by the descent of the annulus. Behind the migrating annulus, the mitochondria accumulate around the axoneme and form the mitochondria sheath at the middle piece (Figure 1B) [15, 16, 19].

In most mammals, including humans, the distal centriole progressively degenerates in mature epididymal sperm, while the proximal centriole is maintained in the mature spermatozoa [20–22]. Recent studies have found that human, bovine, and fruit fly spermatozoa have a typical proximal centriole and a remodeled distal centriole with atypical structure and composition. The discovery indicates that the two centrioles participate in spindle pole formation and play a critical role in fertility and early embryonic development [23–26]. However, rodent sperm lose both the proximal and the distal centrioles when they are fully mature [27, 28] and do not produce a microtubule organizing center or form sperm asters during fertilization. Thus, murine oocytes compensate for the lack of paternal centrosomal contribution in rodents [29, 30], and the mouse zygote forms an acentriolar spindle that is made up of many mini asters [31].

Defects of HTCA formation lead to acephalic spermatozoa

HTCA formation anomalies might lead to different degrees of abnormal spermatozoa. The most extreme form of sperm-neck fragility and teratozoospermia is acephalic spermatozoa syndrome, which results in headless spermatozoa and complete male infertility. Some acephalic spermatozoa may appear to be similar to pin-head spermatozoa [32], but in fact, the sperm head is relatively normal, and it just dislocates from the tail [33]. Some acephalic spermatozoa have round ends with a large cytoplasmic droplet, which are sometimes incorrectly diagnosed as globozoospermia [34, 35]. In the past decades, the typical phenotype of acephalic spermatozoa syndrome has been characterized as familial-clustering, suggesting it is a syndrome with a specific genetic origin [12, 33, 36–39]. Since spermatogenesis in mice is similar to that in humans, and a lot of spermatid-specific expressed genes are conserved, mice provide an important model for understanding the pathogenesis of human acephalic spermatozoa. In mice, the knockouts of several genes led to the production of decapitated spermatozoa with different degrees of HTCA abnormalities (Table 1). Spermatogenesis associated 6 (*Spata6*) was the first gene to be identified that produced nearly 100% headless spermatozoa when it was knocked out in mice. SPATA6 is considered as a part of the pericentriolar materials that are involved in the formation of HTCA by interacting with myosin-based microfilaments. The depletion of SPATA6 protein disrupts the proper formation of sperm segmented columns [40]. Another protein of interest is family with sequence similarity 46, member C (FAM46C), which is a highly conserved noncanonical RNA polyadenylation polymerase. Once this protein was disrupted, abnormal HTCA with incomplete segmented column and capitulum was observed and, finally, resulted in decapitated spermatozoa. The interacting partner of FAM46C still needs to be identified [41]. Also noted is protease, serine 21 (PRSS21) that localizes to the sperm neck region and midpiece. The spermatozoa of the mice lacking PRSS21 display decapitated, angulated, and curled tails [42]. Finally, ornithine decarboxylase antizyme 3 (OAZ3) is essential for the tight connection between the head and the tail of spermatozoa. It has been reported that the protein p12 encoded by *Oaz3* interacts with myosin phosphatase targeting subunit 3 (MYPT3) to modulate the protein phosphatase activity in sperm tails [43, 44].

Although the disruption of a lot many mouse genes results in decapitated sperm head, most of their variants or mutations have not been found to be associated with acephalic spermatozoa. Recently, whole-exome sequencing and Sanger sequencing have been used to identify genetic variants/mutations for acephalic spermatozoa, and the disruption of several genes has been found to be associated with HTCA impairment (Table 2). Sad1 and UNC84 domain containing 5 (*SUN5*) was identified as the first disease-causing gene of acephalic spermatozoa syndrome, and about 47.36% (18/38) cases of human acephalic spermatozoa syndrome could be accounted for by SUN5 variants/mutations in patient cohorts [45–48]. SUN5 localizes on the sperm nuclear envelope during spermiogenesis and later accumulates in the neck region of the spermatozoa [49]. In *Sun5*-knockout mice or in patients with certain SUN5 mutations, the sperm flagellum cannot

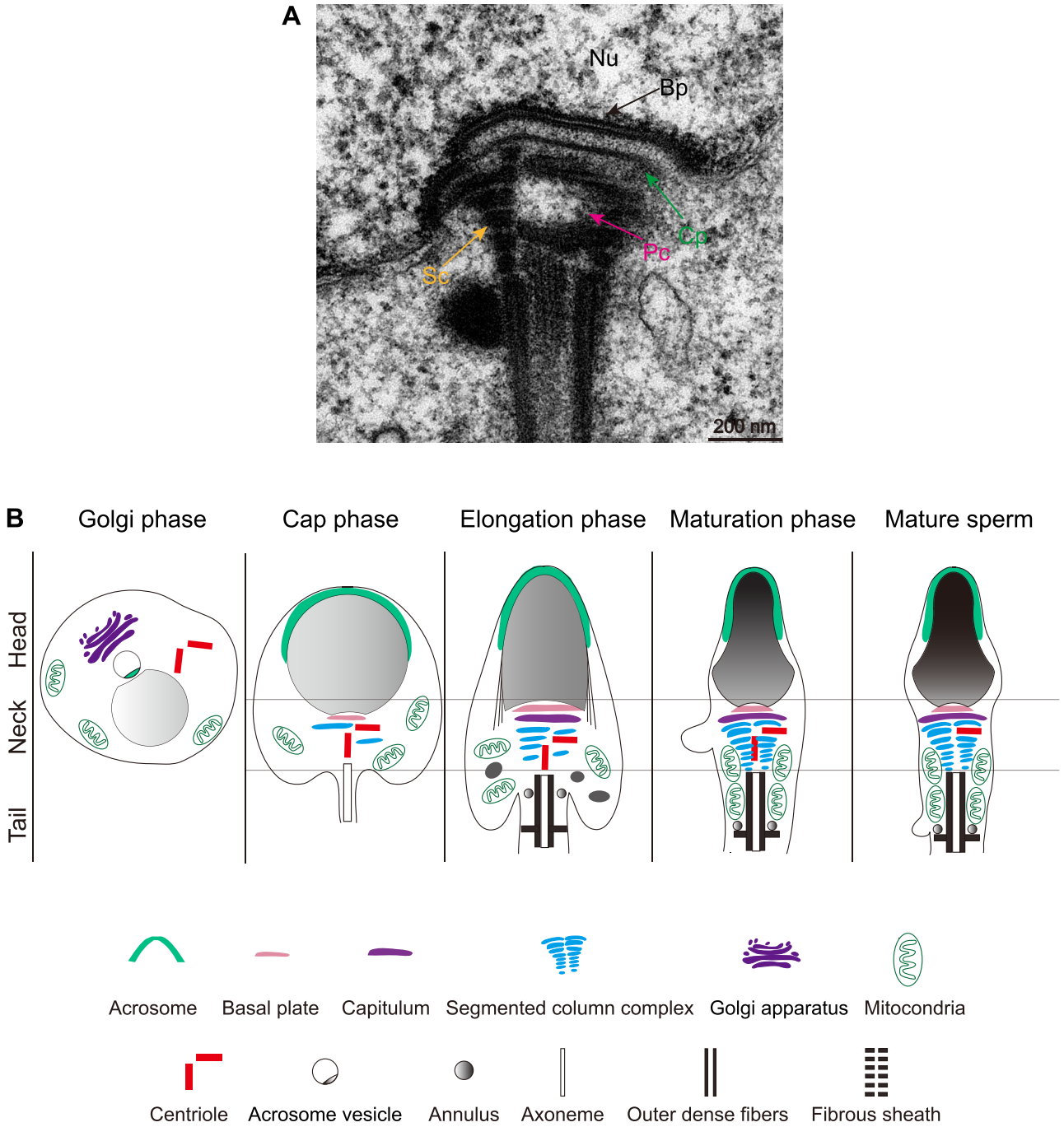

Figure 1. Structural features of mammalian HTCA. (A) Transmission electron microscopy analyses of the mamalian HTCA. The HTCA contains proximal centrioles, distal centrioles, and associated dense material, which includes the capitulum (the dense fibrous plate-like structure) and the segmented columns. Abbreviations: Nu, nuclear; Bp, basal plate; Cp, capitulum; Sc, segmented column; Pc, proximal centriole. (B) Schematic representation of the developmental process of mammalian HTCA. In the Golgi phase, the acrosome vesicle lies in the perinuclear ring, and the centrioles can be observed perpendicular to each other. In the cap phase, the two centrioles migrate to the caudal nuclear pole and expand the electron dense material. The distal centriole begins to form the axoneme. In the elongation phase, Sc formation and capitulum are visible. The manchette and the ring-shaped electron dense granular material of the annulus begin to form. In the maturation phase, the segmented columns and capitulum are fully developed, and the flagellum is surrounded by the mitochondrial sheath, ODFs, and FS. In mature sperm, the distal centriole progressively degenerates.

anchor to the nuclear envelope, and the sperm head separates from the tail during spermiation [45–49]. Polyamine-modulated factor 1-binding protein 1 (*PMFBP1*) is the second validated acephalic spermatozoa-related gene, which is specifically expressed in the testis and localizes to the HTCA. In both humans and mice, the disruption of PMFBP1 results in the separation of sperm heads and tails during spermiogenesis and, finally, leads to the decapitation of the spermatozoa and male infertility [50, 51].

Although SPATA6, SUN5, and PMFBP1 do not physically interact with each other, the three proteins form a sandwich-like structure

Table 1. Mouse models with abnormal HTCA.

KO mouse	Localization	Spermatozoa phenotype	References
Ccdc42	The manchette and base of cilium	Defects in the number and location of the HTCA, lack flagellated sperm	[53, 57]
Cep131	The HTCA	Misalignment of HTCA with the nucleus and displaced implantation fossa, lack flagellated sperm	[62]
Cntrob	The manchette, centrosome, and the marginal ring of the spermatid acroplaxome	Defective acroplaxome marginal ring, detached centrosome, decapitated and disorganized tails	[63]
Fam46c	The manchette	Incomplete segmented column and capitulum	[41]
Hook1	The attachment site of the flagellum to the nucleus and the manchette	Malformed head, elongated manchette, decapitated and curled tails	[56]
Ift88	Proacrosomal vesicles, the HTCA, and the manchette	Malformed HTCA and tail, abnormal head shaping, and ectopic assembled manchette	[59]
Oaz3	The connecting piece, ODFs and the FS	Separation occurred between the basal plate and capitulum	[43, 44]
Odf1	The manchette, ODFs	Decapitated and curled tails, abnormal ODF and mitochondrial sheath, decreased motility	[54, 55]
Pmfbp1	The HTCA	Coupling apparatus detached from the sperm nucleus	[50]
Prss21	The sperm neck region and midpiece	Decapitated, angulated and curled tails, decreased motility	[42]
Spata6	Segmented columns and the capitulum	Lack of the segmented columns and the capitulum, incomplete ODFs and axonemes	[40]
Sun5	Sperm nuclear envelope and the HTCA	Coupling apparatus detached from the sperm nuclear envelope	[49]

Table 2. Genetic mutations/variants in human acephalic spermatozoa syndrome.

Gene	Chromosome location	Mutation sites	References
BRDT	1p22.1	p. Gly928Asp	[83]
HOOK1	1p32.1	p. Gln286Arg	[84]
PMFBP1	16q22.2	p. Tyr109X	[50, 51]
		p. Lys854Argfs*5	
		p. Gln488*	
		p. Gln802*	
		p. Arg909*	
		p. Ala698Profs*7	
SUN5	20q11.21	p. Thr275Met	[45–48, 85]
		p. Arg356Cys	
		p. Met162Lys	
		p. Val128Serfs*7	
		p. Val261Met	
		p. Trp72*	
		p. Asn348Ile	
		p. Ser284*	
		p. Gly114Arg	
		p. Leu143Serfs*30	
		p. Arg159*	
		p. Gln277*	
		p. Arg356His	
		p. Ser71Cysfs*11	
TSGA10	2p11.2	p. Ala71Hisfs*12	[86]

on HTCA [50]. SUN5 acts as the root of the structure, which connects the coupling apparatus to the sperm nuclear envelope. PMFBP1 is located in the middle region between SUN5 and SPATA6 (Figure 2). In *Pmfbp1*- and *Sun5*-knockout mice, ultrastructure

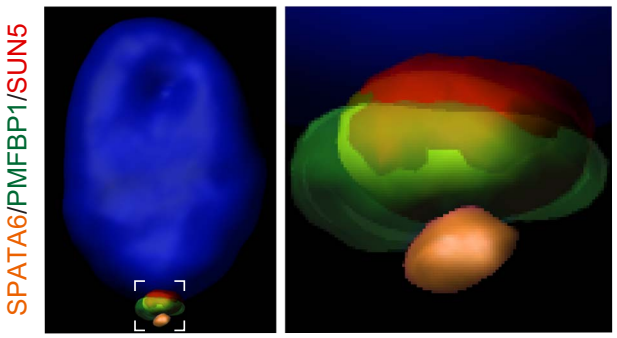

Figure 2. Schematic representation of the relative positions between SUN5, PMFBP1, and SPATA6 in spermatozoa. Single-sperm immunofluorescence analysis of human spermatozoa for SUN5/PMFBP1 and PMFBP1/SPATA6 was performed, respectively. The three-dimensional models were reconstituted by IMARIS software. The axial view is shown of the three-dimensional model, highlighting the relative positions of SUN5, PMFBP1, and SPATA6. The green staining domain is the largest and covers the red domain. SUN5 is shown in red, PMFBP1 is shown in green, and SPATA6 is shown in orange. SUN5 provides the root of the structure, while PMFBP1 is located in the middle region between SUN5 and SPATA6.

analyses of testes show that acephalic spermatozoa have tails with well-assembled HTCA and the separation occurs between the nuclear envelope and basal plate. Analyses of *Spata6*-knockout mice show that the basal plate is implanted in the nucleus, whereas the segmented columns and the capitulum are completely absent or partially formed. However, the depletion of OAZ3 leads to separation between the basal plate and the capitulum [43]. In addition, the segmented columns and the capitulum could be observed in the separated tail of *Oaz3*-knockout mice. Together, these results suggest that OAZ3 might be located between SUN5 and SPATA6, but its relationship to PMFBP1 is still unknown.

The mechanism underlying HTCA formation

One common phenomenon in those well-established acephalic spermatozoa is that impaired HTCAs are always associated with some type of defect in the sperm tail axoneme [40, 49, 50], hinting that the formation of HTCA and flagella might be coupled to each other.

HTCA is an asymmetrical architecture, with the segmented columns connecting to ODFs. The major proteins of the ODFs have been shown to be constituents of the basal belt, capitulum, and segmented columns, and they are likely responsible for structurally connecting the ODFs with the columns and in turn with the capitulum [52]. The small heat shock protein outer dense fiber of sperm tails 1 (ODF1) localizes specifically to the ODFs and functions to correctly arrange the ODFs and mitochondrial sheath. It has been shown that ODF1 localizes to the manchette and sperm tail [53]. In *Odf1*-knockout mice, the sperm head to tail junction is fragile, which leads to a high frequency of sperm head to tail detachment [52, 54, 55]. All these results suggest that ODF1 could participate in HTCA formation, either directly or indirectly.

As a transient microtubule/actin-enriched perinuclear structure, the manchette might also play an important role in HTCA formation. Recent studies have shown that the dysfunction of some proteins located to the manchette leads to abnormal microtubule disassembly and irregular HTCA. For example, the hook microtubule tethering protein 1 (HOOK1) is specifically expressed in male gametes, and it directly interacts with the manchette. Truncated HOOK1 affects manchette removal and the sperm head–tail connection, ultimately leading to decapitated spermatozoa [56]. In addition, coiled-coil domain containing 42 (CCDC42), which is located near the manchette, HTCA, and tail, is important for HTCA formation [53]. Deletion of CCDC42 leads to the multiplicity of the HTCA defects, dislocation of the HTCA, and axoneme assembly defects. However, disruption of CCDC42 did not prevent manchette formation, so it was speculated that CCDC42 might be a cargo protein that is transported along the manchette to the HTCA [57].

Two main cargo transport systems exist in highly polarized spermatids, including the intramanchette transport (IMT) and the intraflagellar transport (IFT). IMT and IFT share similar cytoskeletal components: microtubules and actin-containing microfilaments (F-actin), which provide tracks for cargo protein transportation. IMT might be involved in the nucleus-cytoplasmic transport and the delivery of cargo to the HTCA [58–61]. Centrosomal protein 131 (CEP131) localizes in the HTCA. The loss of CEP131 protein disrupts IMT cargo localization. In *Cep131* mutant spermatids, manchettes are structurally abnormal and the HTCA misaligns with the nucleus and flagella are lacking [62]. Centrobin (CNTROB) is a centrosome protein that is essential for spindle assembly and centrosome duplication. Rats with a hypodactylous (*hd*) mutation, which disrupts CNTROB, showed a disturbance in manchette assembly, incorrect HTCA formation, and structural defects in flagella [63]. In addition, one of the IFT family members, intraflagellar transport 88 (IFT88), localizes to the manchette, HTCA, and tail. Once this protein is disrupted, manchette ectopic assembly and the development of the HTCA are affected. In *Ift88*-mutant mice, spermatids display abnormal head shaping and are tail-less [59]. These results suggest that the disruption of cargo protein transportation might result in sperm decapitation and abnormal tail formation.

In addition, studies have demonstrated that intraflagellar transport 20 (IFT20), kinesin family member 3A (KIF3A), and 27 (KIF27) are transported through the manchette to the basal body region [64–66]. Yet, not all sperm tail proteins localize to the manchette, which leads to the increased possibility that different mechanisms play a role in sperm tail protein storage and transport. It is worth noting that in addition to a potential role in the manchette and cargo transport systems in HTCA formation, the abovementioned proteins might also be involved in the cargo transport systems, the manchette formation, and the HTCA formation independently, and thus, it might have their own roles in these structures or processes.

We know that the HTCA assembles gradually throughout spermiogenesis. Just as construction materials cannot self-organize into a skyscraper and auxiliary facilities are needed to build skyscrapers, HTCA formation requires auxiliary facilities: the assistance of chaperone proteins. Our recent studies have indicated that coupling-apparatus protein DnaJ heat shock protein family (Hsp40) member B13 (DNAJB13) might serve as a protein chaperone that helps SUN5 folding, facilitating its binding to an unknown protein to integrate the sperm head to the tail. Once the HTCA is well-formed, the chaperone protein will be removed from the neck region of the sperm [47]. The chaperone-assisted assembly is just the beginning of our understanding about the molecular mechanism underlying HTCA formation, and more auxiliary facilities for HTCA formation are expected to be found in the near future.

Phylogenetic analysis of various HTCA associated proteins

We found that SUN5 is highly conserved in mammals, and all SUN5 proteins localize to the neck region of spermatozoa [49], suggesting that SUN5 or even the HTCA might be evolutionary conserved in mammals. There are even some case reports about headless spermatozoa in other species, such as bulls, boar, and emu [67–71], suggesting the HTCA might have evolutionarily conserved roles. To better understand the functional role of HTCA and the pathogenic mechanism of headless spermatozoa in different organisms, we analyzed the phylogenetic relationship of SUN5, SPATA6, and PMFBP1 in vertebrates. We downloaded the protein sequences from NCBI (https://www.ncbi.nlm.nih.gov/protein), UniProt (http://www.uniprot.org), and Ensembl (http://asia.ensembl.org/index.html). We then aligned the homologous sequences and constructed multispecies phylogenetic trees of the three proteins using MEGA 10.0 with the Neighbor-Joining (NJ) method. Bootstrap analyses were carried out using 1000 replications with the p-distance model. An online tool was used to display and annotate the phylogenetic trees [72]. According to the phylogenetic trees, we found that the mammalian ortholog of these three proteins was all clustered into a single subgroup, while their homologues in bird, reptile, amphibian, and bony fish were clustered into two to three subgroups (Figure 3), suggesting that the components of HTCA might be evolutionarily conserved in mammals but diversified in other species.

The evolution of the sperm neck region

The diversity of the components of HTCA should be related to their structures and functions in sperm competition. In the 1950s, Ake Franzen proposed that the spermatozoa's form and function should be affected by the mode and environment of fertilization. Species that use external fertilization have sperm with a less elaborate morphology than those using internal fertilization, which potentially reflects an adaptation to the more complex female reproductive

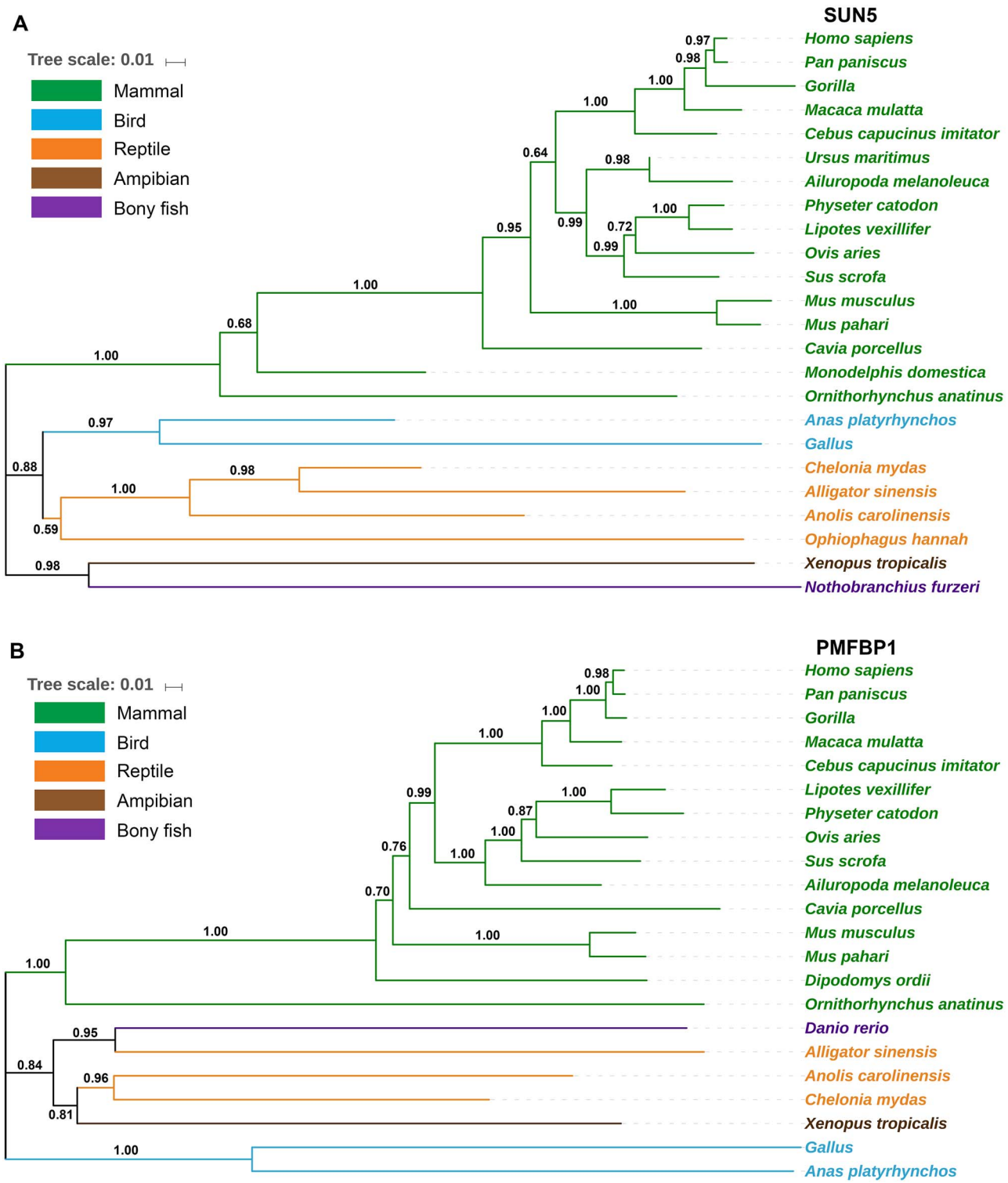

Figure 3. Multiple species phylogenetic trees of HTCA associated proteins. (A) Multiple species phylogenetic tree of SUN5. (B) Multiple species phylogenetic tree of PMFBP1. (C) Multiple species phylogenetic tree of SPATA6. The phylogenetic trees were constructed using MEGA 10.0 with the NJ method. We performed 1000 bootstrap replicates. The trees were divided into five groups, and colors were used to represent different groups, with mammal in green, bird in blue, reptile in orange, amphibian in brown, and bony fish in purple.

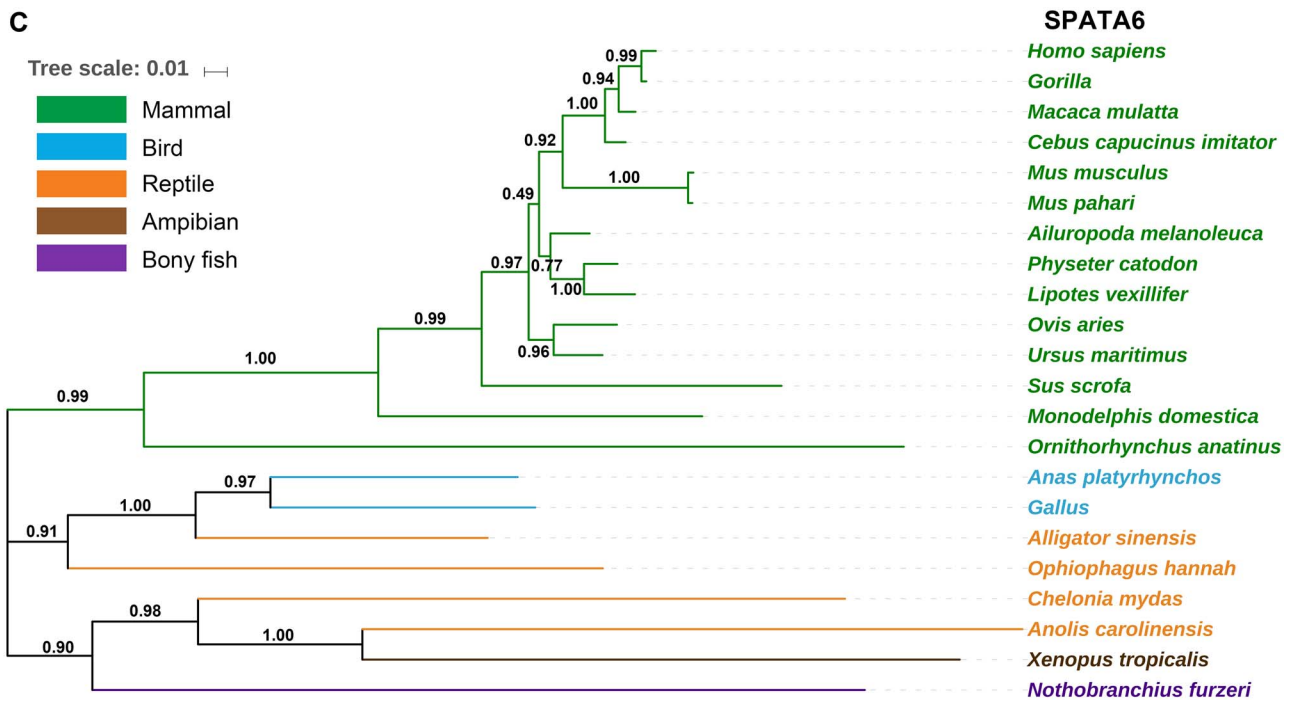

Figure 3. Continued.

tract [73, 74]. In aquatic vertebrates such as some fishes, the males discharge their spermatozoa into the water for fertilization, and their spermatozoa are relatively short with a large cellular body consisting of a head, neck, and a thin vibrating tail [75]. Most of the amphibians also use external fertilization (*Xenopus* for example); their spermatozoa are thin vermiform cells and have a simple neck structure consisting of a pair of centrioles [76]. With the transition from aquatic to terrestrial, internal fertilization has been evolved to adapt to the environment. The spermatozoa of terrestrial vertebrates are much more complex and have strengthened and lengthened flagellum. In reptiles, the turtle spermatozoon shows a neck that consists of a connecting collar of dense material and extends to the implantation fossa at the base of the nucleus. The flagellum originates from the distal centriole, containing the accessory fibers ODFs, FS, and the mitochondrial sheath [77]. Bird spermatozoa have some unique characteristics; the neck of fowl spermatozoa is short and contains dense material that originates from the two perpendicular centrioles, which is implanted in the base of the head nuclear membrane [78]. Unlike mammalian sperm flagellum, the prominent outermost dense fibers, which surround the nine doublet fibers in the tails of mammalian, are absent from the spermatozoa of the fowl. The principal piece of the flagellum of fowl spermatozoa consists of a sheath of dense amorphous material deposited continuously around the axoneme (Figure 4). The unique structure of fowl spermatozoon might be related to its fertilization through the cloaca, where spermatozoa are transferred from male to female. In mammalian spermatozoon, the neck is usually defined as the constriction between the head and the middle part of the mitochondrial sheath and is made up of nine distinct segmented columns that combine with ODFs at the tail [79]. It has been proposed that the motility of the spermatozoa is partly regulated by the HTCA. HTCA initiates the flagella waveforms and maintains the flagella to oscillate [80, 81]. Together, the neck of most vertebrates' spermatozoa has a specialized dense material that surrounds the caudal end of the nucleus, and the intercentriolar material of the terrestrial spermatozoon might function as a mechanical connection to reinforce the integration of the sperm head and tail [82].

Conclusions and future perspectives

The HTCA is a centrosome-based structure consisting of two perpendicular centrioles and associated components, and the formation of HTCA is an intricate and complex process. The transportation of cargo proteins that relies on microfilament and microtubule systems might be involved in the HTCA formation.

Defects in SUN5, PMFBP1, SPATA6, and some other genes result in impaired HTCA formation, lead to the detachment of sperm heads from their tails, and finally result in acephalic spermatozoa syndrome and complete male infertility. Most components of the HTCA are evolutionary conserved in mammals but are diversified in other vertebrates. The specialized neck structures may be associated with internal fertilization and might help to facilitate the fertilization of an egg in the long narrow female reproductive tract of most terrestrial vertebrates.

Although some pathogenic mechanisms leading to decapitated spermatozoa have been uncovered, these mechanisms do not explain all forms of acephalic spermatozoa syndrome and further investigation is definitely needed. Because researchers are lacking an *in vitro* spermiogenic system, the mechanisms of HTCA formation are largely speculative, and direct evidence about the functional role of the cargo transport system in HTCA formation is urgently needed. The relationship between the proximal and distal centrioles in HTCA formation also needs to be further addressed. As a structural or functional diversified coupling apparatus, the identification of more HTCA-specific components is expected, but how to identify these

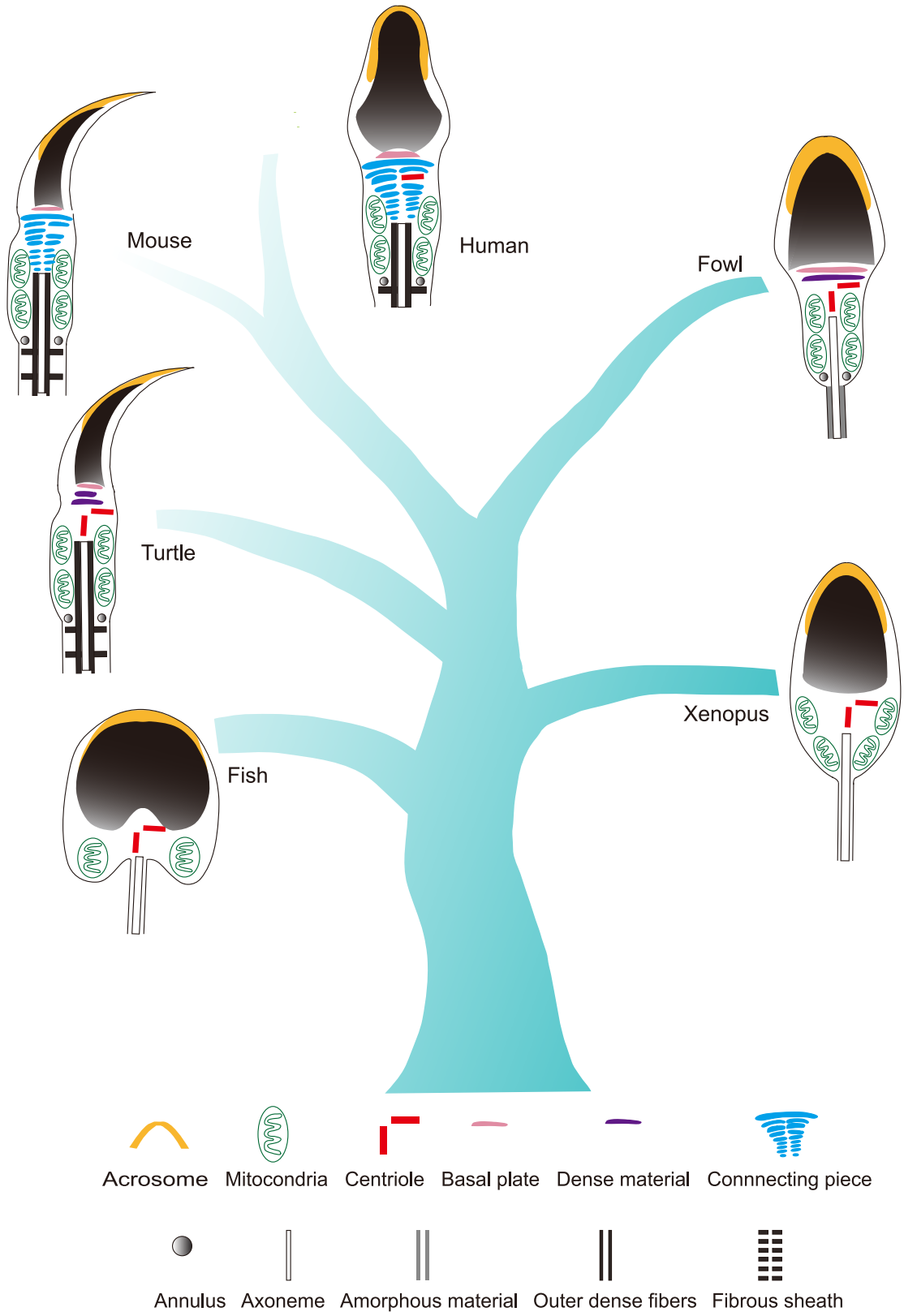

Figure 4. The evolution of the sperm neck structure in different species. In fish, the sperm neck only contains the centrosome. In *xenopus*, the sperm neck contains the centrosome and some mitochondria. In turtle, the sperm neck consists of the connecting collar of dense material. The flagellum is surrounded by the mitochondrial sheath, ODFs, and FS. In fowl, the sperm neck is short and contains the dense material, and the flagellum consists of a sheath of dense amorphous material. In mouse and human, the spermatozoa have the segmented column and capitulum. Note that the mitochondria are only used to show the relative position but not the real numbers.

new components is still a challenge within the field. As new technologies are developed, the relationship between HTCA components and their fine structures is anticipated to become clearer in the near future. Addressing these questions will not only uncover the mystery of HTCA formation but may also provide new ways to diagnose or even treat male infertility caused by acephalic spermatozoa symptom.

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Conflict of interest

The authors declare no competing interests.

Author Contributions

BW created Figures 1B, 3, and 4 and wrote the manuscript; HG and CL created Figures 1A and 2 and edited all the figures; WL initiated and wrote the manuscript.

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