Blackwell Publishing

Asian J Androl 2007; 9 (4): 445–452
DOI: 10.1111/j.1745-7262.2007.00307.x

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·*Review*·

Protein phosphatase PP1γ2 in sperm morphogenesis and epididymal initiation of sperm motility

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Abstract

The serine/threonine phosphatase (PP1) isoform PP1γ2, predominantly expressed in the testis, is a key enzyme in spermatozoa. High PP1γ2 catalytic activity holds motility in check in immature spermatozoa. Inhibition of PP1γ2 causes motility initiation in immature spermatozoa and motility stimulation and changes in flagellar beat parameters in mature spermatozoa. The PP1γ2 isoform is present in all mammalian spermatozoa studied: mouse, rat, hamster, bovine, non-human primate and man. We have now identified at least four of its regulatory proteins that regulate distinct pools of PP1γ2 within spermatozoa. Our studies provide new insights into biochemical mechanisms underlying development and regulation of sperm motility. We hypothesize that changes in sperm PP1γ2 activity as a result of phosphorylation and reversible binding of the regulatory proteins to the catalytic subunit are critical in the development and regulation of motility and the ability of sperm to fertilize eggs. Targeted disruption of the *Ppp1cc* gene, which encodes the PP1γ1 or PP1γ2 isoforms, causes male infertility in mice as a result of impaired spermiogenesis. Our observations suggest that, in addition to motility, the protein phosphatase PP1γ2 might play an isoform-specific function in the development of specialized flagellar structures of mammalian spermatozoa. *(Asian J Androl 2007 July; 9: 445–452)*

Keywords: protein phosphatase; epididymis; sperm motility; spermatogenesis

1 Introduction

The spermatozoon is a highly differentiated cell designed to carry out its special function. The spermatozoon is composed of a head containing the acrosome and a condensed nucleus, and a flagellum consisting of a middle piece, a principal piece and an end piece. Flagellar beat propels spermatozoa through the female reproductive tract and through the external investments of the egg prior to fertilization. Spermatozoa leaving the testis are immotile and cannot fertilize or bind to eggs. Testicular spermatozoa acquire motility and fertilizing ability during their passage through the epididymis.

The epididymis can be divided anatomically into at least four segments: the initial segment, the caput, the corpus, and the caudal epididymidis. Secretions from these segments are composed of distinct proteins. Specific modifications are thought to occur in spermatozoa as they pass through these segments. Spermatozoa removed from the caput epididymidis are immotile and infertile, whereas spermatozoa from the caudal region possess forward motility and the ability to bind and fertilize an egg. Several modifications occur during the passage of sperm through the epididymis. These include remodeling of the sperm plasma membranes, changes in composition and localization of proteins, acquisition and alteration of glycoproteins, and changes in membrane lipid composition [1–3]. The relationship between these changes and the acquisition of sperm motility is not known.

The levels of the intracellular factors known to be

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involved in regulation of kinetic activity of the flagellum, calcium ions (Ca^{2+}), pHi and cyclic adenosine monophosphate (cAMP), change during epididymal sperm maturation [1, 4–7]. The levels of calcium fall, whereas intracellular pH and cAMP levels rise. The capacity for motility already exists in immotile testicular and epididymal sperm because motility can be induced in demembranated immotile spermatozoa [1, 8, 9]. Therefore, it is believed that changes in the intracellular levels of the mediators, cAMP, H^+ and calcium, are responsible for initiation of motility. The mediators are thought to regulate flagellar activity through changes in protein phosphorylation.

The steady-state phosphorylation status of a protein is determined by the relative activities of the protein kinases and phosphatases acting on it. Increases in sperm protein phosphorylation have been implicated in the regulation of sperm function. Research in sperm protein phosphorylation, until recently, was largely focused on protein kinases: protein kinase A (PKA) in particular [10–14]. It is well known that motility stimulation can be affected by cAMP-mediated PKA activation [13, 15, 16]. A role for PKA necessarily implies a function for a protein phosphatase. Protein phosphatases can significantly modify and restrict PKA action. Inclusion of protein phosphatases in the reactivation media prevents motility initiation in demembranated sperm [17, 18]. Inhibition of phosphatase activity results in initiation and stimulation of motility [19, 20], suggesting that phosphatases have an important role in regulation of sperm kinetic activity. Although the role of protein phosphatases as components of signalling and regulatory pathways in other cell types is well known, relatively little is known about phosphatases in spermatogenesis and spermatozoa.

Eukaryotic protein phosphatases are classified into two distinct gene families: serine threonine/phosphatases (PPP) and phosphotyrosine phosphatases (PTP) [21]. Protein phosphatase 1 (PP1) belongs to a family of protein phosphatases, PPP, which include PP1, PP2A and PP2B (calcineurin). The serine/threonine phosphatase, protein phosphatase 1 (PP1), is a highly conserved protein in all eukaryotes. It controls a variety of processes, such as cell division, transcription, translation, muscle contraction, glycogen and lipid metabolism, neuronal signalling and embryonic development [22–24]. In mammals, there are four catalytic subunit isoforms of PP1, encoded by three genes: PP1α, PP1β, PP1γ1 and PP1γ2 [22, 25, 26]. The enzymes PP1γ1 and PP1γ2, alternatively spliced variants generated from a single gene [26, 27], are identical in all respects except that PP1γ2 has a unique 21-amino-acid carboxy-terminus extension. Although PP1α, PP1β and PP1γ1 are ubiquitous, PP1γ2 is predominantly expressed in the testis and appears to be the only PP1 isoform in spermatozoa [19, 20]. Targeted disruption of the *Ppp1cc* gene, resulting in the loss of PP1γ1 and PP1γ2, causes infertility in male mice as a result of impaired spermiogenesis [26, 28]. This indicates that one or both of the isoforms are involved in sperm development and possibly spermiation (the release of mature spermatozoa from the seminiferous epithelium into the lumen). It is intriguing that the other isoforms of PP1, owing to their high level of amino acid sequence conservation, are able to substitute for the absence of PP1γ1 and PP1γ2 in all tissues except the testis.

Sperm formation in mammals is characterized by a well-defined sequence of mitotic and meiotic divisions, followed by a long period of complex morphogenetic differentiation, leading to the production of mature spermatozoa [29]. Mammalian sperm development, taking place in the seminiferous tubules of the testis, can be divided into three distinct stages: proliferative, meiotic (spermatogenesis) and spermiogenic (post-meiotic differentiation and morphogenesis). Although previous reports have shown that PP1γ2 is the predominant PP1 isoform expressed in the testis [19, 20], its distribution within specific cell types in the murine testis relative to the other PP1 isoforms is unknown. Using isoform-specific antibodies against PP1γ1, PP1γ2 and PP1α, a distinct differential distribution of these proteins in wild-type testicular cells and spermatozoa from mice was observed [30]. To elucidate further the involvement of PP1 isoforms in spermatogenesis, we have also analyzed the morphology of developing spermatids from *Ppp1cc*-null mice. Our results indicate that PP1γ2 loss has a profound effect on sperm structure and morphogenesis.

This review briefly summarizes our present understanding of PP1γ2 and its regulation in mammalian spermatozoa in terms of motility. In addition to motility, we also suggest a role of PP1γ2 in development of specialized flagellar structures of mammalian spermatozoa.

2 Expression of PP1γ2 in the testes and spermatozoa and its role in motility

Enzyme activity and Western blot analyses showed that PP1γ2 is a predominant serine/threonine protein phosphatase in testes [19, 20]. Various murine tissue extracts analyzed by Western blots showed that PP1γ2 is a testis-predominant isoform (Figure 1). Western blot analysis also showed that all three PP1 isoforms were present in murine testes [30]; whereas PP1γ2 was the only PP1 isoform detected in spermatozoa. These observations in murine spermatozoa and testes are identical to those found with bovine, human and rhesus monkey spermatozoa [19, 20, 31]. Expression profiles of PP1

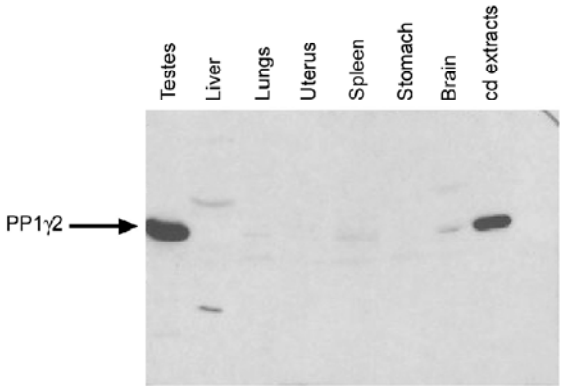

Figure 1. Immunoreactive PP1γ2 in mouse tissues. Protein extracts from testes, liver, lung uterus, spleen, stomach, brain and caudal epididymal sperm were separated by SDS/PAGE, transferred on to nitrocellulose membrane, and probed with PP1γ2 antibody antibody. 50 μg of protein was loaded per lane with the exception of caudal sperm extracts where 20 μg was used.

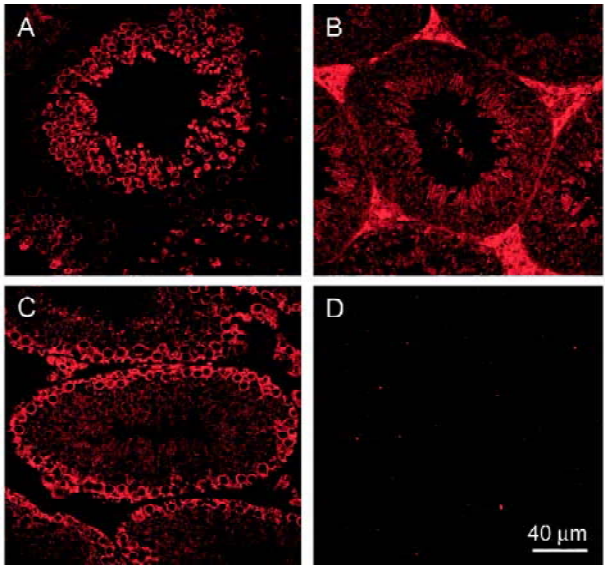

Figure 2. Localization of PP1γ1, PP1γ2 and PP1α in wild-type mouse testes sections. (A): PP1γ2 is prominently expressed in the cytoplasm of germ cells ranging from secondary spermatocytes and round spermatids to elongating spermatids and spermatozoa. (B): PP1γ1 expression is observed predominantly in the interstitial cells of Leydig. (C): PP1α showed strong expression in interstitial cells, spermatogonia, peritubular cells and pachytene spermatocytes. (D): No signal was seen when preimmune sera was used alone. Bar = 40 μm.

isoforms in various cell types of the adult testis of wild-type mice using immunohistochemistry demonstrated that PP1γ2 was the only isoform abundant in secondary spermatocytes, round and elongating spermatids (Figure 2A), whereas PP1γ1 and PP1α were restricted to spermatogonia, pachytene spermatocytes and interstitial cells (Figures 2B and 2C). Postnatal expression of PP1γ2 in developing testes increases with age [30]. The distinct expression patterns of PP1α, PP1γ1 and PP1γ2 during post-natal testicular development and spermatogenesis suggest non-overlapping roles for the PP1 isoforms in cells within the testis.

Furthermore, antibodies against the PP1γ2 carboxy-terminus showed that the protein was present in spermatozoa from a wide range of mammalian species: immunoreactive PP1γ2 was detected in murine, hamster and bovine spermatozoa (Figure 3A), but was absent from *Xenopus* spermatozoa. The enzyme PP1γ2 is likely to be present in all mammalian spermatozoa. Surprisingly, the carboxy-terminus of PP1γ2, which is not essential for its catalytic activity [32], is conserved, as judged from Western blot analysis. In contrast, PP1γ1 which is present in *Xenopus* spermatozoa, is absent from mammalian spermatozoa (Figure 3B). Sperm from sea urchin and turkey contain immunoreactive PP1 resembling PP1α (data not shown).

High protein phosphatase activity is correlated with low sperm motility, whereas low catalytic activity is associated with vigorous motility in bovine and monkey spermatozoa [19, 20, 31]. Soluble extracts of caput epididymidal spermatozoa contain significantly higher PP1γ2 activity than caudal epididymidal spermatozoa, in

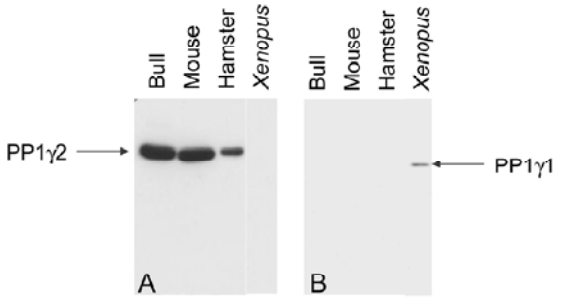

Figure 3. Immunoreactive PP1γ2 and PP1γ1 in spermatozoa from different species. Sperm extracts (20 μg in each) were subjected to SDS-PAGE followed by Western blot analysis with PP1γ2 (A) and PP1γ1 (B) antibodies.

extracts containing the same amount of immunoreactive PP1γ2 [19, 20]. These data suggest that the high catalytic activity of PP1γ2 might be responsible for the lack of motility in immature caput epididymidal spermatozoa. This suggestion is further supported by the observation that inhibition of protein phosphatase activity by calyculin A and okadaic acid induces motility in caput spermatozoa [20]. A lowering of PP1γ2 catalytic activity might be part of biochemical mechanisms underlying sperm motility development in the epididymis. Protein phosphatases in general are regulated by their binding and

targeting proteins [22, 25]. Following identification of PP1γ2, we expected that one or more somatic cell protein regulators of PP1 might be present in spermatozoa. PP1γ2 in extracts from caput and caudal epididymidal spermatozoa can be chromatographically resolved into several distinct pools. The proteins associated with PP1γ2 in these chromatographic fractions were identified by micro-sequencing.

3 Protein regulators of sperm PP1γ2

3.1 Mammalian homologue of the yeast PP1 regulatory protein: sds22

One of the proteins that co-eluted with PP1γ2 in immuno-affinity chromatography and was identified by micro-sequencing of sperm extracts was sds22 [33]. A homologue of this protein was identified as a PP1 binding protein in yeast and a nuclear protein in mammalian somatic cells. The enzyme PP1γ2 bound to sds22 is catalytically inactive against the substrate phosphorylase *a* [33], a situation analogous to sds22-bound yeast PP1 [34]. Intriguingly, however, unlike in yeast and somatic cells, a substantial portion, if not all, of sperm sds22 is cytoplasmic [33]. It appears that PP1γ2-sds22 binding is regulated. What regulates sds22 binding to PP1γ2 is not yet known. Phosphorylation of sds22 or some intermediary protein might be involved [35]. It is possible that a cycle of PP1γ2 activation and inactivation, owing to its binding to and dissociation from sds22, might be part of the biochemical mechanisms regulating motility and other sperm functions. Studies are in progress to determine how the biochemical mechanisms underlying PP1γ2-sds22 binding regulate sperm function.

3.2 Protein 14-3-3

Protein 14-3-3 isoforms are a highly conserved family of acidic proteins, expressed in a variety of eukaryotic cells. They bind to a wide variety of proteins to regulate and coordinate several cellular processes, such as cell cycle progression, apoptosis, protein trafficking, cytoskeleton rearrangements, metabolism and transcriptional regulation of gene expression [36–38]. The effect of 14-3-3 binding depends on the nature of its ligand. Binding might activate or inhibit the enzyme activity or change the localization and phosphorylation status of proteins [39]. More than 100 14-3-3 binding partners have been identified in somatic cells through affinity chromatography coupled with proteomic analysis [40–43]. We first documented the expression of 14-3-3 in mature spermatozoa and showed that it binds to a distinct pool of PP1γ2 in spermatozoa [44]. It appears that PP1γ2 bound to 14-3-3 is phosphorylated. The physiological significance of this binding is yet to be unraveled. It is possible that 14-3-3 regulates PP1γ2 catalytic activity, phosphorylation or its interaction with other proteins. There is also evidence that, in addition to PP1γ2, at least three other 14-3-3 binding phosphoproteins exist. The identities of these proteins is not known [44]. Studies are underway to identify those and other 14-3-3 binding phosphoproteins and the biological relevance of their binding to PP1γ2. Protein 14-3-3 is present in spermatozoa isolated from species as diverse as *Xenopus*, turkey, mouse, bull and man, suggesting an essential role for this protein in male gamete function.

3.3 Inhibitor 2 (I2) and glycogen synthase kinase-3 (GSK-3)

Our studies on sperm PP1γ2 first focused on identification of heat-stable inhibitors of the enzyme. The first candidate protein examined was the ubiquitously expressed PKA-regulated inhibitor 1 (I1). The established role of PKA in sperm function made I1 a logical candidate. Surprisingly, I1 activity was undetectable in bovine sperm extracts; however, substantial activity resembling inhibitor 2 (I2) was present in heat-stable sperm extracts [20, 45]. This activity was thought to be I2-like because inhibition could be reversed by glycogen synthase kinase-3 (GSK-3). We have now used specific antibodies to confirm the presence of inhibitor I2 in spermatozoa (Figure 4). Spermatozoa contain high levels of GSK-3 activity and GSK-3 is significantly less phosphorylated

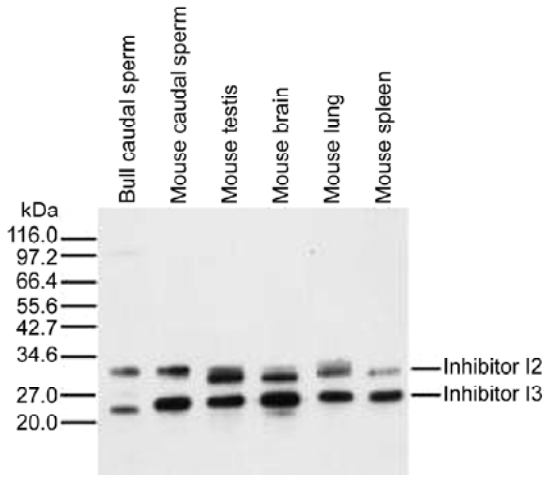

Figure 4. Presence of inhibitor 2 (I2) and inhibitor 3 (I3) in testes and spermatozoa. Heat stable tissue and sperm extracts following Western blot were probed with affinity purified PPP1R11 (I3) antibody (1:1 000) raised against the whole I3 protein (a gift from Dr Mathieu Bollen, Belgium), and affinity purified peptide I2 antibody (1:1 000) raised against amino acid residues 134–147, EKKRQFEMKRKLH, of I2.

(i.e. more active) in immotile caput compared to motile caudal epididymidal spermatozoa [20, 45]. Furthermore, an increase or decrease in motility causes a corresponding increase or decrease in tyrosine and serine phosphorylation of GSK-3 [46]. It appears that GSK-3 is inactivated by a combination of tyrosine and serine/threonine phosphorylation. The upstream GSK-3-regulating enzymes, PI3-kinase, PDK1 and PKB, are also present in spermatozoa [46]. It is likely that one of the consequences of inactive GSK-3 (e.g. in caudal spermatozoa) is the lowering of PP1γ2 activity because inhibitor I2 is likely to be unphosphorylated and, hence, able to bind to PP1γ2. Therefore, low GSK-3 and PP1γ2 activities might be prerequisites for the optimum function of spermatozoa. However, the question of whether an extracellular signal activates sperm GSK-3 remains to be answered.

3.4 Inhibitor 3 (I3)

A potent heat-stable PP1 inhibitor was identified through yeast two-hybrid studies designed to identify PP1-binding proteins from human brain [47]. This protein is identical to the protein product of human hemochromatosis candidate gene *HCG V*, orthologous to the mouse *Ppp1r11*, also called *Tctex5*. This is localized to the *t* complex, a naturally occurring polymorphism of the proximal third of chromosome 17 and represented by a family of closely related *t* haplotypes that carry similar mutant alleles of genes implicated in sperm function [48]. The heavily mutated *t*-allele of *Ppp1r11* is thought to code for one of three tightly linked *t* haplotype proteins whose expression in sperm from *t/t* men coincides with a flagellar waveform phenotype, "curlicue", which is strongly associated with the sterility of these men [49–51]. The protein product of HCG V/Tctex5 is a protein phosphatase 1 regulatory subunit 11 (PPP1R11, TCTEX5, inhibitor I3). Like protein phosphatase inhibitor I1 and inhibitor I2, PPP1R11 is hydrophilic, heat stable, behaves anomalously on SDS-PAGE and is a specific PP1 inhibitor [47]. Additionally, I3 is extremely sensitive to the protease activity.

We found that PPP1R11 is ubiquitously expressed in various murine tissues and highly expressed in bovine and murine testis and spermatozoa (Figure 4); PPP1R11 from murine and bovine spermatozoa is bound to PP1γ2 both *in vitro* and *in vivo*, as shown by immunoprecipitation (IP), microcystin-agarose and GST/His-tagged recombinant-I3 proteins pull down assays. Protein phosphatase assays showed that PPP1R11 inhibited the catalytic activity of both recombinant PP1γ2 and PP1γ2 in sperm extracts, and that PP1γ2 bound to the GST-fusion protein in the pull-down assays was catalytically inactive. Studies are underway to determine the role of inhibitor I3 in sperm motility.

4 Is the PP1γ2 isoform essential in spermatozoa?

The isoforms PP1γ1 and PP1γ2 are alternatively spliced products of a single gene consisting of eight exons [26, 27]. The two isoforms result from the inclusion (PP1γ1) or exclusion (PP1γ2) of an intron between exons 7 and 8. This last exon contributes the unique 21-amino-acid extension in PP1γ2. This carboxy-terminus sequence does not appear to be essential for catalytic activity, as truncated PP1γ2 lacking the 21-amino-acid C-terminus is able to complement the PP1-deficient yeast cell [32]. Male mice lacking the PP1γ gene (i.e. both PP1γ1 and PP1γ2 isoforms) are sterile owing to arrest

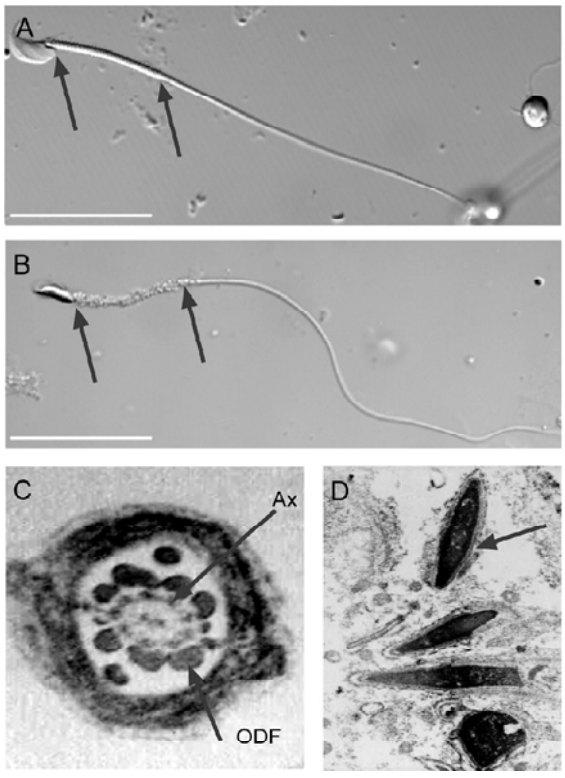

Figure 5. Aberrant morphology of testicular spermatozoa of *Ppp1cc*-null mouse. (A): Normal hook shape head and mid-piece of testicular spermatozoa of wild type mouse. Scale bar = 40 μm. (B): *Ppp1cc*-null sperm display mid-pieces that have disorganized mitochondrial sheaths and aberrant sperm head structure. Scale bar = 40 μm. (C): Transverse section through the principal piece of -/- developing sperm tail showing many extra outer dense fibers (ODF) (total of 13) and apparent developmental abnormality of the fibrous sheath, particularly the morphology of its longitudinal columns (× 80 000). (D): An example of degeneration of a condensing spermatid from a -/- mouse (× 80 000). Ax, axonemal complex.

of spermatogenesis at the spermatid stage [26, 28, 52]. In contrast, *Ppp1cc*-null females are all fertile [26]. There was a significant reduction in spermatids and testicular spermatozoa in *Ppp1cc*-null testes. Loss of spermatids occured at the round spermatid stage and increased in severity resulting in a marked reduction in condensing and elongating spermatids and an almost complete absence of mature spermatozoa. Intriguingly, PKA knockout and casein kinase knockout mouse models do not show this phenotype. Therefore, our results suggest that PP1γ2, along with an unknown protein kinase, might be involved in protein phosphorylation and dephosphorylation events during spermiogenesis.

The reduced number of spermatozoa in *Ppp1cc*-null mice might be a result of either increased apoptosis or cell death or a result of a defect in spermiation [30] leading to phagocytic activity of Sertoli cells [53, 54]. A detailed ultrastructural analysis using light and transmission electron microscopy showed numerous structural defects in elongating spermatids and testicular spermatozoa of the *Ppp1cc*-null male mice (Figure 5). Abnormal head shapes were also observed in agreement with a previous report [28]. Prominent defects observed were poorly developed or missing mitochondrial sheaths, and supernumerary, disorganized outer dense fibers (ODF) throughout the sperm tails [30]. We also detected frequent degeneration of condensing spermatids, indicated by fragmentation of tail structures and the presence of numerous vacuoles in the cytoplasm of elongating spermatids. A subtle abnormality in the development of the fibrous sheath was observed, specifically in the formation of distinct, triangular-shaped longitudinal columns and inward projections that replaced the ODF associated with axonemal microtubule doublets 3 and 8 in wild-type sperm [30]. These observations suggest that PP1γ2 is required for flagellar integrity and the development of flagellar structures. Defects in flagellar structures prompted us to determine the expression of some of the post-meiotic proteins. Western blot analysis and immunohistochemistry showed the absence of, or a sharp reduction in, the levels of post-meiotic proteins (AKAP4/AKAP82, odf2, sds22, FSII) in *Ppp1cc*-null testes [30]. Some of these proteins appear to be associated with sperm tail development and function [20, 33, 55–57]. This reduction of protein expression could be a result of a lack, or reduced number, of cell types expressing these proteins or a result of reduced protein expression in spermatids. Although studies using selected antibodies and immumnofluorescence in sections suggest reduced protein expression in the null testes, further examination using immunocytochemistry of testicular spermatozoa has shown that AKAP4, odf2, FSII and sds22 are present in mutant spermatozoa [30]. This suggests that the apparent reduction in intracellular protein levels seen in Western blots could be a result of a reduced number of differentiating spermatids in the null testis where these proteins are synthesized. It is possible that defective tail development might be a result of reduced levels of these flagellar proteins. Additional studies are required to determine the exact role of PP1γ2 in protein synthesis in developing spermatids.

Our experiments show that the PP1γ2 isoform is present in all mammalian spermatozoa studied (mouse, hamster and bull), and absent from non-mammalian species, such as *Xenopus* (Figure 3A). In contrast, PP1γ1 is present in *Xenopus* sperm extracts (Figure 3B). Therefore, it is tempting to speculate that PP1γ2 might have an isoform-specific function in the development of outer dense fibers and the fibrous sheath, which are structures found in mammalian sperm. If this is true, then PP1γ2, but not PP1γ1, should restore sperm formation and fertility in *Ppp1cc*-null mice. Moreover, because spermatozoa lack PP1γ1, it is more likely that lack of PP1γ2 is responsible for the morphological defects in spermatozoa. To test this hypothesis, we made transgenic mice expressing either PP1γ1 or PP1γ2 under the testis-specific Pgk2 promoter. Studies to determine which of the two isoforms might rescue the phenotype in *Ppp1cc*-null mice are underway in our laboratory.

In summary, the enzyme PP1γ2 is a key signalling protein in spermatozoa. The enzyme is regulated in novel ways: by phosphorylation and by proteins identified for the first time in spermatozoa. Understanding how regulation of the enzyme is essential in spermatogenesis and in mature sperm function has implications for clinical andrology and in the identification of novel targets for the development of male contraceptives.

Acknowledgment

We thank Dr Stephen Pilder (Temple University, PA, USA) for the transmission electron microscopy and Dr Mike Model (Department of Biological Sciences, Kent State University, Kent, OH, USA) for his assistance in confocal fluorescence microscopy. This work was supported by grants from the National Institutes of Health (HD38520, USA).

References

1 Bedford JM, Hoskins DD. The mammalian spermatozoon: morphology, biochemistry and physiology. In: Lamming GE, editor. Marshall's Physiology of Reproduction. Edinburgh, London, Melbourne, New York: Churchill Livingstone; 1990: 379–568.

2 Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E, Neill J, editors. The Physiology of Reproduction. New York: Raven Press; 1988.
3 Jones R. Plasma membrane structure and remodelling during sperm maturation in the epididymis. J Reprod Fertil Suppl 1998; 53: 73–84.
4 Cooper TG, Yeung CH. Physiology of sperm maturation and fertilization In: Nieschlag E, Behre HM, editors. Andrology, Male Reproductive Health, Dysfunction. Berlin: Springer; 1997: 61–78.
5 Vijayaraghavan S, Bhattacharya A, Hoskins DD. Calcium uptake by bovine epididymal spermatozoa is regulated by the redox state of the mitochondrial pyridine nucleotide. Biol Reprod 1989; 40: 744–51.
6 Vijayaraghavan S, Hoskins DD. Regulation of bovine sperm motility and cyclic adenosine 3',5'-monophosphate by adenosine and its analogues. Biol Reprod 1986; 34: 468–77.
7 Vijayaraghavan S, Critchlow LM, Hoskins DD. Evidence for a role for cellular alkalinization in the cyclic adenosine 3',5'-monophosphate-mediated initiation of motility in bovine caput spermatozoa. Biol Reprod 1985; 3: 489–500.
8 Mohri H, Yanagimachi R. Characteristics of motor apparatus in testicular, epididymal and ejaculated spermatozoa. Exp Cell Res 1980; 127: 191–6.
9 Lindemann CB. A cAMP-induced increase in the motility of demembranated bull sperm models. Cell 1978; 13: 9–18.
10 Burton KA, Muller CH, Dunphy EL, McKnight GS. Deletion of type II alpha regulatory subunit delocalizes protein kinase A in mouse sperm without affecting motility or fertilization. J Biol Chem 1999; 274: 24131–6.
11 Burton KA, McKnight GS. PKA, germ cells, and fertility. Physiology 2007; 22: 40–6.
12 Nolan MA, Babcock DF, Wennemuth G, Brown W, Burton KA, McKnight GS. Sperm-specific protein kinase A catalytic subunit Calpha2 orchestrates cAMP signalling for male fertility. Proc Natl Acad Sci U S A 2004; 101:13483–8.
13 Vijayaraghavan S, Goueli SA, Davey MP, Carr DW. Protein kinase A-anchoring inhibitor peptides arrest mammalian sperm motility. J Biol Chem 1997; 272: 4747–52.
14 Garbers DL, Kopf GS. The regulation of spermatozoa by calcium cyclic nucleotides. Adv Cyclic Nucleotide Res 1980; 13: 251–306.
15 San Agustin JT, Witman GB. Role of cAMP in the reactivation of demembranated ram spermatozoa. Cell Motil Cytoskeleton 1994; 27: 206–18.
16 Esposito G, Jaiswal BS, Xie F, Krajnc-Franken M, Robben TJ, *et al*. Mice deficient for soluble adenylyl cyclase are infertile because of a severe sperm-motility defect. Proc Natl Acad Sci U S A 2004; 101: 2993–8.
17 Takahashi D, Murofushi H, Ishiguro K, Ikeda J, Sakai H. Phosphoprotein phosphatase inhibits flagellar movement of triton models of sea urchin spermatozoa. Cell Struct Funct 1985; 10: 327–37.
18 Murofushi HIshiguro K, Takahashi D, Ikeda J, Sakai H. Regulation of sperm flagellar movement by protein phosphorylation and dephosphorylation. Cell Motil Cytoskeleton 1986; 6: 83–8.
19 Smith GD, Wolf DP, Trautman KC, da Cruz e Silva EF, Greengard P, Vijayaraghavan S. Primate sperm contain protein phosphatase 1, a biochemical mediator of motility. Biol Reprod 1996; 3: 719–27.
20 Vijayaraghavan S, Stephens DT, Trautman K, Smith GD, Khatra B, da Cruz e Silva EF, *et al*. Sperm motility development in the epididymis is associated with decreased glycogen synthase kinase-3 and protein phosphatase 1 activity. Biol Reprod 1996; 54: 709–18.
21 Barford D, Das AK, Egloff MP. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu Rev Biophys Biomol Struct 1998; 27: 133–64.
22 Ceulemans H, Bollen M. Functional diversity of protein phosphatase 1, a cellular economizer and rest button. Physiol Rev 2004; 557: 1–8.
23 Wang PJ, Chien MS, Wu FJ, Chou HN, Lee SJ. Inhibition of embryonic development by microcystin-LR in zebrafish, Danio rerio. Toxicon 2005; 45: 303–8.
24 Wera S, Hemmings BA. Serine/threonine protein phosphatases. Biochem J 1995; 311: 17–29.
25 Cohen PT. Protein phosphatase 1– targeted in many directions. J Cell Sci 2002; 115: 241–56.
26 Varmuza S, Jurisicova A, Okano K, Hudson J, Boekelheide K, Shipp EB. Spermiogenesis is impaired in mice bearing a targeted mutation in the protein phosphatase 1c gamma gene. Dev Biol 1999; 205: 98–110.
27 Kitagawa Y, Sasaki K, Shima H, Shibuya M, Sugimura T, Nagao M. Protein phosphatases possibly involved in rat spermatogenesis. Biochem Biophys Res Commun 1990; 171: 230–5.
28 Davies T, Varmuza S. Development to blastocyst is impaired when intracytoplasmic sperm injection is performed with abnormal sperm from infertile mice harboring a mutation in the protein phosphatase 1cgamma gene. Biol Reprod 2003; 4: 1470–6.
29 Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev 1972; 52: 198–236.
30 Chakrabarti R, Douglas K, Jing L, Joanne O, Stephen P, Vijayaraghavan S. Analysis of *Ppp1cc*-null mice suggests a role for PP1γ2 in sperm morphogenesis. Biol Reprod 2007; 76: 992–1001.
31 Smith GD, Wolf DP, Trautman KC, Vijayaraghavan S. Motility potential of macaque epididymal sperm: the role of protein phosphatase and glycogen synthase kinase-3 activities. J Androl 1999; 1: 47–53.
32 Okano K, Heng H, Trevisanato S, Tyers M, Varmuza S. Genomic organization and functional analysis of the murine protein phosphatase 1c gamma (*Ppp1cc*) gene. Genomics 1997; 1: 211–5.
33 Huang Z, Khatra B, Bollen M, Carr DW, Vijayaraghavan S. Sperm PP1γ2 is regulated by a homologue of the yeast protein phosphatase binding protein sds22. Biol Reprod 2002; 67: 1936–42.
34 MacKelvie SH, Andrews PD, Stark MJ. The Saccharomyces cerevisiae gene sds22 encodes a potential regulator of the mitotic function of yeast type 1 protein phosphatase. Mol Cell Biol 1995; 15: 3777–85.
35 Mishra S, Somanath PR, Huang Z, Vijayaraghavan S. Binding and inactivation of the germ cell-specific protein phosphatase PP1gamma2 by sds22 during epididymal sperm maturation. Biol Reprod 2003; 5: 1572–9.
36 Gardino AK, Smerdon SJ, Yaffe MB. Structural determinants of 14-3-3 binding specificities and regulation of subcellular localization of 14-3-3-ligand complexes: a comparison of the X-ray crystal structures of all human 14-3-3 isoforms. Semin Cancer Biol 2006; 3: 173–82.
37 Aitken A. 14-3-3 proteins: a historic overview. Semin Cancer Biol 2006; 16: 162–72.
38 Yaffe M. Master of all things phosphorylated. Biochem J 2004; 379: e1–2.
39 Bridges D, Moorhead GB. 14-3-3 proteins: a number of functions for a numbered protein. Sci STKE 2005; 295: re10.
40 Milne FC, Moorhead G, Pozuelo Rubio M, Wong B, Kulma A, Harthill JE, *et al*. Affinity purification of diverse plant and human 14-3-3-binding partners. Biochem Soc Trans 2002; 30: 379–81.
41 Jin J, Smith FD, Stark C, Wells CD, Fawcett JP, Kulkarni S, *et al*. Proteomic, functional, and domain-based analysis of *in vivo* 14-

3-3 binding proteins involved in cytoskeletal regulation and cellular organization. Curr Biol 2004; 14: 1436–50.
42 Meek SE, Lane WS, Piwnica-Worms H. Comprehensive proteomic analysis of interphase and mitotic 14-3-3-binding proteins. J Biol Chem 2004; 279: 32046–54.
43 Pozuelo Rubio M, Geraghty KM, Wong BH, Wood NT, Campbell DG, Morrice N, *et al*. 14-3-3-affinity purification of over 200 human phosphoproteins reveals new links to regulation of cellular metabolism, proliferation, and trafficking. Biochem J 2004; 379: 395–408.
44 Huang Z, Myers K, Khatra B, Vijayaraghavan S. Protein 14-3-3zeta binds to protein phosphatase PP1gamma2 in bovine epididymal spermatozoa. Biol Reprod 2004; 1: 177–84.
45 Vijayaraghavan S, Mohan J, Gray F, Khatra B, Carr D. A role for phosphoryaltion of glycogen synthase kinase-3alpha in bovine sperm motility regulation. Biol Reprod 2000; 6: 1647–54.
46 Somanath PR, Jack SL, Vijayaraghavan S. Changes in sperm glycogen synthase kinase-3 serine phosphorylation and activity accompany motility initiation and stimulation. J Androl 2004; 25: 605–17.
47 Zhang J, Zhang L, Zhao S, Lee EY. Identification and characterization of the human HCG V gene product as a novel inhibitor of protein phosphatase-1. Biochemistry 1998; 37: 16728–34.
48 Lyon MF. Transmission ratio distortion in mouse *t*-haplotypes is due to multiple distorter genes acting on a responder locus. Cell 1984; 37: 621–8.
49 Olds-Clarke P, Johnson LR. *t*-haplotypes in the mouse compromise sperm flageller function. Dev Biol 1993; 155: 14–25.
50 Olds-Clarke P. Sperm from tw32/+ mice: capacitation is normal, but hyperactivation is premature and nonhyperactivated sperm are slow. Dev Biol 1989; 131: 475–82.
51 Lindemann CB, Goltz JS, Kanous KS, Gardner TK, Olds-Clarke P. Evidence for an increased sensitivity to Ca^{2+} in the flagella of sperm from tw32/+mice. Mol Reprod Dev 1990; 26: 69–77.
52 Jurisicova A, Lopes S, Meriano J, Oppedisano L, Casper RF, Varmuza S. DNA damage in round spermatids of mice with a targeted disruption of the Pp1cgamma gene and in testicular biopsies of patients with non-obstructive azoospermia. Mol Hum Reprod 1999; 5: 323–30.
53 Russell LD, Hikim APS, Ettlin RA, Clegg ED. Histological and histopathological evaluation of the testis. Clearwater, FL: Cache River Press, 1990.
54 Nakanishi Y, Shiratsuchi A. Phagocytic removal of apoptotic spermatogenic cells by Sertoli cells: mechanisms and consequences. Biol Pharm Bull 2004; 27:13–16.
55 Miki K, Willis WD, Brown PR, Goulding EH, Fulcher KD, Eddy EM. Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. Dev Biol 2002; 248: 331–42.
56 Schalles U, Shao X, van der Hoorn FA, Oko R. Developmental expression of the 84 kDa ODF sperm protein: localization to both the cortex and medullar outer dense fibers and to the connecting piece. Dev Biol 1998; 199: 250–60.
57 Carr DW, Fujita A, Stentz CL, Liberty GL, Olson GE, Narumiya S. Identification of sperm-specific proteins that interact with a-kinase anchoring proteins in a manner similar to the type ii regulatory subunit of PKA. J Biol Chem 2001; 276: 17332–8.