Adaptive Evolution of Primate Sperm Proteins

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Adaptive pressures throughout primate evolution have shaped sperm and seminal fluid proteins, causing them to change more rapidly than most other proteins. The adaptations are thought to be in response to sperm competition, sexual conflict, pathogen evasion and reinforcement.

Advanced article

Article Contents

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Introduction

Proteins involved in sexual reproduction evolve more rapidly when compared to other classes of proteins. For example, when researchers compare protein sequences between primate species, sperm proteins as a class are among the most rapidly evolving (Nielsen *et al*., 2005; Gibbs *et al*., 2007). This observation is puzzling when one considers the importance of reproduction to the persistence of the species, because rapid evolutionary changes may jeopardize the coordinated steps of mating and fertilization. However, the importance of these proteins could be the reason behind their rapid evolution. Mounting evidence suggests that a large proportion of their amino acid changes were beneficial and resulted from sexual selection.

Adaptive evolution describes the fixation of beneficial mutations in a population through the process of positive Darwinian selection. Adaptation is of general interest because it reveals functionally important changes and contributes to our understanding of biological processes. However, one cannot infer adaptive evolution based solely on rapid divergence between species because a lack of functional constraint can also cause a protein's primary sequence to evolve rapidly, as for a pseudogene. A mutation at a pseudogene should carry no functional consequence, detrimental or beneficial, so it is said to be selectively neutral. Several tests of adaptive evolution evaluate a gene's deoxyribonucleic acid (DNA) sequence for characteristics expected after positive selection. To infer adaptive evolution, these characteristics must be more extreme than patterns created by selective neutrality. **See also**: Molecular Evolution; Molecular Evolution: Neutral Theory

There are two general forms of DNA sequence data used to test for adaptive evolution, polymorphism within a species and divergence between species. Polymorphic data consist of genetic differences between individuals in a population. Positive selection at a specific gene is tested by comparing the patterns of polymorphisms in the gene region to the distribution expected from neutral evolution and the population's history. Divergence between species is often used to test for adaptive evolution by comparing the numbers of amino acid changes between species to silent changes in the coding sequence. Under selective neutrality, rates of amino acid and silent substitutions (d_N and d_S, respectively) are expected to be equal, yielding a d_N/d_S ratio of 1. A significant excess of amino acid changes indicates that recurrent positive selection altered the amino acid sequence of the protein, yielding a d_N/d_S ratio greater than 1. It is important to remember that these two types of data, polymorphism and divergence, provide information on different time scales. Polymorphism reveals the recent history of a population, which for primates is typically tens of thousands of years. Divergence data represent the major trend over an evolutionary period separating species, which can be over millions of years for primate species. For a review of tests of selection as applied to humans (see Sabeti *et al*., 2006). **See also**: Neutrality and Selection in Molecular Evolution: Statistical Tests; Synonymous and Nonsynonymous Rates

Sperm pass several steps to obtain their singular goal of fertilization. They are produced in the testis through the process of spermatogenesis and are stored in the epididymis. After mating, sperm must pass the cervix, move through the uterus and wait for an egg at the entrance of the fallopian tubes, all the while under attack by the female immune system. During the journey from ejaculate to egg, sperm are also accompanied by several factors from seminal fluid that provide them with an energy source, partial protection from immune attack and mechanisms to compete with sperm from other males. At the time of ejaculation, sperm are not prepared to fertilize an egg, and they must go through the steps of capacitation, during which they undergo changes in motility and receptivity to egg binding. Sperm are at least partially guided to an egg by chemotaxis to follicular fluid. Once an egg is found, a sperm increases its swimming activity, a state termed hyperactivation. Using hyperactivity a sperm passes through an outer gelatinous layer (cumulus layer) and then binds to the

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more substantial egg coat called the zona pellucida in mammals. After recognizing a female egg coat protein (ZP3), the sperm releases proteins stored in its acrosome, an organelle found at the front tip of the sperm head. These proteins open a hole in the zona pellucida, allowing the sperm head to pass and fuse with the egg membrane. As will be discussed later, only a few proteins functioning in these steps are known. **See also**: Reproduction in Eutherian Mammals; Sperm–Egg Interactions: Sperm–Egg Binding in Mammals

Several sperm proteins performing the steps above evolve under strong adaptive pressures. Often, it is difficult to assign the force driving their adaptive evolution, but some cases have been explained through careful examination of behaviour and protein function. Other important clues are gained through analogy to adaptive pressures in nonprimate species. Such studies lead to exciting discoveries about the pressures that shape reproduction in our own species through their influence on our fertility and behaviour.

Forces Driving Adaptation of Reproductive Proteins

Adaptive evolution among reproductive proteins is found across diverse taxonomic groups, including plants, molluscs, insects and vertebrates (Clark *et al*., 2006). The phenomenon may be universal to sexually reproducing organisms, and the types of pressures driving adaptive evolution are likely shared between these groups. There are several forces hypothesized to drive the adaptive evolution of reproductive proteins, many of which are likely to affect primate proteins as well.

Sexual selection and sperm competition

Sexual selection prefers traits that provide an individual with more successful matings and fertilizations, especially in comparison with individuals of the same sex. Importantly, these are traits that influence reproduction rather than survival. Sexual selection explains the divergence of conspicuous traits such as courtship behaviour and sexual ornamentation, and it can operate equally well at the microscopic level of gametes. If an egg preferentially binds a certain allele of a sperm protein, assortative mating occurs. For example, some sea urchins eggs prefer sperm carrying a particular allele of a sperm protein, while some eggs prefer a different allele (Palumbi, 1999). This observation is consistent with sexual selection. **See also**: Sexual Selection

Sperm competition can occur when a female mates with multiple males during a single ovulatory period. The females of several primate species are known to mate with multiple males (polyandry) which creates the conditions for sperm competition and sexual selection on sperm. In nonprimate species, which are amenable to experimentation, competitive advantages have been observed in which one mate sires a disproportionate amount of offsprings. Sperm competition predicts continuous adaptive evolution with an intensity comparable to the degree of polyandry in a species. Competitive differences in sperm could exist over swimming, chemotaxis, egg coat penetration and egg membrane fusion. Sperm competition could also drive adaptation in seminal fluid proteins, such as those that form copulatory plugs. **See also**: Postcopulatory Reproductive Strategies

Sexual conflict

Reproduction is largely cooperative between the sexes, but there is also conflict over parental investment. Reproductive traits of each sex will evolve to provide the maximum benefit to that sex, but there are several traits for which the optima differ between males and females. One example is a conflict over the rate of fertilization. As sperm competition increases the rate of fertilization, the risk of more than one sperm fertilizing the egg (polyspermy), which stops development, also increases. Both females and males suffer a loss of offspring, but sperm competition will continue to exert evolutionary pressure because faster sperm will tend to win more nonpolyspermic fertilizations. Consequently, female traits would counter-evolve to return the rate to a moderate level, creating a coevolutionary chase between male and female characters. Sexual conflict could be a powerful driving force among reproductive proteins. **See also**: Sexual Conflict

Reinforcement

After a period of geographic separation, populations of a species may become differentially adapted to their particular environments. When these populations become reunited they may reassume mating and form hybrids. However, such hybrids might be less fit for several reasons, either related to their environments or to their internal genetic interactions. The result is that producing hybrids would be a less than ideal use of gametes because they are less likely to survive and produce offspring in further generations. Therefore, reproductive barriers to block interpopulation mating and fertilization would be beneficial. The selective pressure to create such barriers is called reinforcement. Reinforcement can act to reduce mate attraction between the populations, or it can introduce incompatibilities in genitalia or sperm–egg interactions. **See also**: Reinforcement

Pathogen defence

Sperm must pass through both the male and female reproductive tracts, neither of which is devoid of microorganisms. Studies of human males have found that the presence of sexually transmitted pathogens decreases sperm count and quality (Bezold *et al*., 2007). An evolutionary response would be to mount defences to these pathogens. Several insect species transport antibacterial proteins in their ejaculates, and human seminal fluid contains proteins that

demonstrate antibiotic activity. However, pathogens commonly evolve to defeat such defences, spurring further adaptive evolution of the semen proteins. The result is a classical host–pathogen arms race. **See also**: Coevolution: Host–Parasite

Sperm Proteins

Sperm have developed specialized proteins to aid in their quest to reach and fertilize an egg. Several of these proteins have evolved adaptively during primate evolution (**Figure 1**).

Motility

The popular image of sperm racing in formation towards the egg may not be entirely accurate, but it provides a convenient illustration of one aspect of sperm competition. Males with faster sperm and sperm that better regulate energy usage should tend to win more fertilizations. Consistent with this idea, studies of human populations have found signs of recent selective sweeps encompassing genes that mediate motility, *SPAG4*, *ODF2* (Voight *et al*., 2006) and *SPAG6* (Williamson *et al*., 2007). Additional evidence is required to confirm that these sweeps were caused by selection on sperm, but they suggest selective events during the recent evolution of our species. Another sperm trait that could mediate competition is ability to find an egg. Human sperm chemotax to follicular fluid (Ralt *et al*., 1991). Although no sperm proteins involved in chemotaxis are known, sperm chemotaxis could be another arena of competition in primate reproduction.

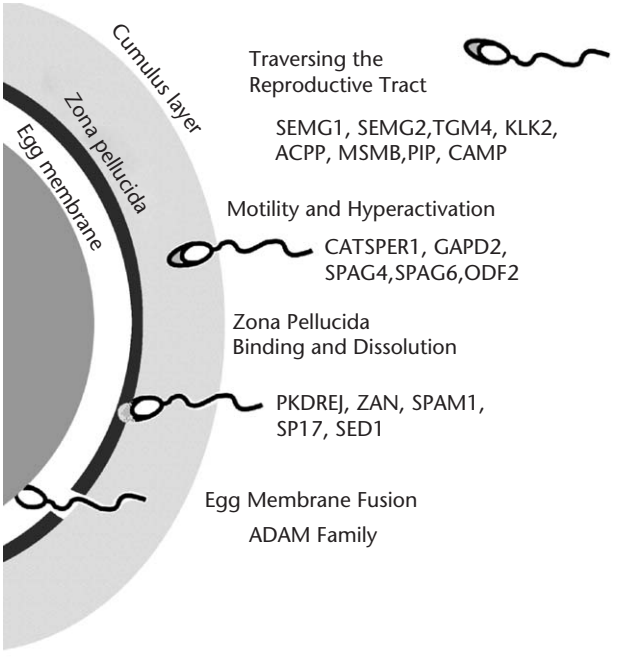

Figure 1 Steps of fertilization and specific proteins showing adaptive evolution.

Sperm require a great deal of energy to swim through the female reproductive tract. The sperm-specific protein, glyceraldehyde 3-phosphate dehydrogenase-2 (GAPD2), helps produce adenosine triphosphate (ATP) for swimming sperm through glycolysis. Its mouse orthlogue, Gapds, is required for sperm motility and fertility (Miki *et al*., 2004). GAPD2 has an additional proline-rich region that nonsperm GAPD proteins do not. This region shows an excess of amino acid changing substitutions when comparing human and rodent sequences (Torgerson *et al*., 2002). These amino acid changes could have been selected because they promote the efficient use of energy or the ability to produce large amounts of ATP at the proper time. Alternatively, coevolution of binding surfaces with other proteins in the sperm tail could drive its divergence because proline-rich domains are thought to mediate protein–protein interactions.

Primate sperm become motile at the time of ejaculation, but upon reaching the egg they become hyperactivated. This drastic change is required to penetrate the egg cumulus layer, and it is triggered by the influx of calcium ions into the sperm tail. Evidence suggests that the four CATSPER proteins form a calcium ion channel and facilitate hyperactivation (Qi *et al*., 2007). CATSPER1 demonstrates a unique pattern of adaptive evolution (Podlaha and Zhang, 2003). Its *N*-terminus has experienced a large number of insertions and deletions during primate (and rodent) evolution. In fact, the number of in-frame indels in CATSPER1 is highly anomalous when compared to the rate at noncoding regions genome-wide. Positive selection for changes in protein length can explain this observation. Interestingly, the *N*-terminus of CATSPER1 is intracellular and is thought to regulate the activity of the ion channel through a ball-and-chain mechanism in which the chain flexes to open and close the ion channel. The lengthening and shortening of the regulatory region may fine-tune the cue for sperm hyperactivation and provide an advantage to competing sperm.

Fertilization

Eggs present substantial barriers to fertilization, such as the zona pellucida, requiring specialized sperm proteins. After hyperactivated sperm traverse the cumulus layer and bind to the zona pellucida they release the contents of their acrosome. Acrosomal proteins then open a hole in the zona pellucida, and the sperm fuses with the egg plasma membrane. Multiple sperm proteins from these steps show evidence of positive selection. It is easy to imagine sperm competition driving their rapid evolution, but there is also evidence of a sexual conflict.

ZP3 is the zona pellucida protein that triggers mammalian sperm to open their acrosomes. Strangely, ZP3 is under positive selection to diversify its amino acid sequence precisely in the regions that sperm recognize (Swanson *et al*., 2001). The adaptive evolution of ZP3 could be attributed to avoidance of pathogens or to a sexual conflict over the rate of fertilization, as described earlier. In the case of conflict,

adaptive diversification of ZP3 changes the sperm-binding site, slowing the fertilization reaction to protect eggs from polyspermy. Proteins from competing sperm would then adaptively coevolve to 'catch-up' and bind better to the changing ZP3 surface, providing an opportunity to observe coevolution between females and males. Unfortunately, there is no consensus over which sperm protein recognizes ZP3. Several candidates have been proposed, each with its own evidence. Some of these candidates show signs of positive selection in primates and mammals, such as PKDREJ, SPAM1, sperm protein 17 (SP17), SED1 and ZAN.

During primate evolution both PKDREJ and Zonadhesin (ZAN) experienced a significant excess of amino acid changes ($d_N/d_S > 1$), a sign of adaptive evolution (Gasper and Swanson, 2006; Hamm *et al*., 2007). The PKDREJ protein belongs to a family responsible for interactions with extracellular matrix; one member of this family, suREJ, triggers the acrosome reaction in sea urchin sperm. The PKDREJ sites under positive selection in primates are in the extracellular region, making egg interactions a likely force behind adaptation. Interestingly, human amino acid polymorphisms in PKDREJ are found mostly within this region (Hamm *et al*., 2007). ZAN is a large protein containing several domains implicated in cell–cell interactions. Its sites under selection are found in multiple regions, and they also correspond well with sites of human amino acid polymorphism. A good example of ZAN's rapid evolution is found in its large 13th exon; it has diverged more than the introns of the same gene. This exon also contains small repeats characteristic of interaction-mediating mucin domains. Another form of evidence implicates ZAN in sperm competition. In primate species in which males are larger than females (sexual dimorphism), it is thought that males compete physically with one another and that sperm competition is less important. ZAN's evolution in primates is consistent with this prediction; it has evolved slower in species with greater size differences between the sexes (Herlyn and Zischler, 2007).

Other zona pellucida-binding candidates have been examined by comparing human sequences with those of several other mammals. Such comparisons can highlight adaptive evolution over a broad time scale, but it is difficult to say whether it extends into primate evolution specifically without more primate sequences. SP17 shows evidence of binding the zona pellucida in rabbits, and demonstrates strong signs of positive selection when comparing mammalian species (Swanson *et al*., 2003). In similar comparisons, SPAM1 (or PH20) shows positive selection predominantly in its *C*-terminal region which is thought to interact with the zona pellucida (Torgerson *et al*., 2002; Swanson *et al*., 2003). Yet another protein proposed to facilitate zona pellucida binding is SED1. It experienced a burst of adaptive evolution during the evolutionary lineage leading to primates. During this period it lost a notch-like epidermal growth factor (EGF) domain and experienced the adaptive fixation of several amino acid changes (Podlaha *et al*., 2006). The dynamic evolutionary histories of these zona-binding candidates is consistent with roles in sexual selection or reinforcement.

Evolutionary information can guide reproductive research. Clearly, a major challenge in the study of primate reproduction is to determine the sperm proteins which bind the zona pellucida. Perhaps the sperm receptor question can best be answered through a combination of genetic, biochemical and coevolutionary evidence (Swanson *et al*., 2003). There could also be several weakly binding sperm proteins that trigger the acrosome reaction, involving redundancy (Castle, 2002). Moreover, the rapid evolution of fertilization proteins may make comparisons between divergent mammalian groups tenuous. Binding partners in rodents might be completely different from those in ruminants or primates.

Some proteins from the ADAM family (A Disintegrin And Metalloprotease) are expressed in the testis and are implicated in sperm–egg membrane binding. Among the ADAM proteins expressed in reproductive tissues, ADAMs 1, 2 and 32 show signs of positive selection (Torgerson *et al*., 2002; Civetta, 2003; Swanson *et al*., 2003; Glassey and Civetta, 2004). As with zona-binding proteins above, the sites under positive selection in ADAMs 1 and 2 are overrepresented within the egg-binding region, consistent with sperm–egg coevolution driving the rapid evolution of these proteins. There could be more cases of adaptive evolution during sperm–egg fusion waiting to be discovered, but the proteins mediating this step in primates are only now being discovered. Recent reports show that a sperm protein, Izumo, is required for sperm–egg fusion.

Species specificity and speciation

Barriers to heterospecific fertilization have been observed across several taxonomic groups including mammals. The fact that both sperm and egg receptors are evolving rapidly could only accelerate the creation of these barriers. Sexual selection, conflict and sperm competition, all processes inherent to the reproductive system, could contribute to reproductive isolation. When geographically separated (allopatric) populations are reunited, their sperm–egg binding proteins might have diverged and become less compatible, which may lead to a speciation event. Alternatively, reinforcement predicts that the benefit of forming barriers to hybridization is an evolutionary force driving the divergence of these proteins. These distinct hypotheses are difficult to resolve with the current evidence. Primates have strong pre-mating barriers to cross-species reproduction, such as visual cues, so that reproductive isolation may not require barriers at fertilization. However, rapid sperm–egg evolution could contribute to allopatric speciation of isolated populations. **See also**: Speciation: Allopatric; Speciation: Introduction

Seminal Fluid Proteins

The primate ejaculate contains seminal fluid proteins that perform various functions ranging from protection to competition. These proteins originate in the seminal

vesicles, prostate, epididymis and bulbourethral glands. Five seminal fluid proteins show antibacterial activity and are also produced in general secretory glands to protect the ducts and secreted factors from infection. Of these, prolactin-induced protein (PIP), beta-microseminoprotein (MSMB) and cathelicidin (CAMP) have evolved under positive selection within primates (Clark and Swanson, 2005; Zelezetsky *et al*., 2006). Transferrin and its homologue lactotransferrin inhibit bacterial growth by sequestering iron from plasma; lactotransferrin and transferrin were shown to evolve adaptively in primates and vertebrates, respectively (Ford, 2001; Gibbs *et al*., 2007). The selective pressure driving these proteins is probably a coevolutionary arms race with invasive bacteria. Bacteria develop ways to block their action, and the antibacterial protein changes its structure to escape that block. Sperms are also threatened by the female immune system because they are foreign cells in the female reproductive tract. In fact, seminal fluid contains high concentrations of prostaglandins that can downregulate the cellular immune response, presumably to protect sperm (Kelly and Critchley, 1997). Prostate-specific transglutaminase 4 (TGM4) is also proposed to protect sperm from immune attack by altering and cloaking the sperm surface. Since prostaglandins are lipid molecules their evolution is not easily studied, but the protein TGM4 has two independent sources of evidence for adaptive evolution. In a study of human populations encompassing the entire genome, the region containing the *TGM4* gene was implicated in a recent positive selective sweep, suggesting that a recent change in the gene was beneficial (Voight *et al*., 2006). Also, the TGM4 coding sequence showed positive selection for amino acid diversification among primate species (Clark and Swanson, 2005). TGM4 is an exciting case because both polymorphism and divergence evidence point to its importance in human evolution. Its functional study could shed light on human reproductive issues such as infertility.

A competitive function of seminal proteins is to form a copulatory plug, which in some primate species is a barrier to sperm from subsequent mates. The firmness of semen coagulum varies greatly among primate species, but it has been shown to form a firm plug in species that compete postmating, such as chimpanzees (Dixson and Anderson, 2002). Chimpanzee females mate with several males around the time of ovulation. At the other extreme, semen remains in a liquid state in species that do not compete after mating, such as gorillas. The gorilla mating system is typified by a physically dominant, 'silverback' male that has exclusive access to females. Since most females mate only with the silverback, competition between males would be through physical confrontation rather than sperm competition. The presence of a copulatory plug also correlates with predictors of sperm competition, like relative testis size. The proteins that form the coagulum are semenogelins 1 and 2 (SEMG1 and SEMG2). Interestingly, SEMG1 and SEMG2 show signs of positive selection, both between primate species and within populations (Jensen-Seaman and Li, 2003; Kingan *et al*., 2003). Paralleling the correlations of copulatory plugs with competition, the strength of positive selection along species branches for SEMG2 correlates with the degree of polyandry currently in that species (Dorus *et al*., 2004). TGM4 crosslinks the SEMG monomers into a polymer gel. TGM4 was discussed in the preceding paragraph, and its dual roles make its adaptive evolution difficult to interpret. Seminal fluid also contains proteins that breakup copulatory plugs, allowing subsequent males to compete for fertilizations. Of the proteins taking part in dissolution, kallikrein 2 (KLK2) and prostatic acid phosphatase (ACPP) are under positive selection (Clark and Swanson, 2005). KLK2 may have coevolved with a changing substrate because its sites under selection are clustered around its protease active site. Possibly there is an intergenic conflict between proteins that form the coagulum and those that break it down. Each male with more effective dissolution proteins or more resistant plug formation proteins has an advantage. This could lead to a coevolutionary arms race as seen for sexual conflict and host–pathogen interactions.

Just as positive selection supports their implied role in sperm competition, the loss of these proteins in other species also suggests competition. Copulatory plug genes have been lost on several independent occasions during primate evolution. As presented in **Table 1**, different genetic lesions have disabled proteins which form the coagulum, namely SEMG and TGM4. Species that have lost these proteins likely no longer form coagulum or copulatory plugs. Similarly, those proteases that break down coagulum, prostate-specific antigen (PSA) and KLK2, have disappeared or become pseudogenes in multiple lineages. This phenomenon is observed across diverse groups of primate species. It is seen in New World monkeys, such as the tamarin and marmoset, and in apes and Old World monkeys, such as the gorilla, gibbon and macaque. A hypothesis to explain these independent events is that these species do not compete postmating and no longer need a copulatory plug for sperm competition. The absence of sperm competition in the gorilla has been proposed before, and gibbons are known for being at least socially monogamous. Could this also be the case with marmosets and tamarins? Or did they develop other strategies for competition? Two observations are clear; there is both adaptive evolution and high gene turnover among these seminal fluid genes.

Spermatogenesis

Spermatogenesis, the production of sperm, involves proteins regulating germ cells and contributing to the development of sperm. Several of these proteins have also undergone adaptive evolution, although for different reasons. Most spermatogenesis proteins do not enter the female reproductive tract and hence are not driven by coevolution with female-derived proteins.

Sperm carry their genetic material wrapped around small proteins called protamines. There is a stark contrast between the evolution of protamines and their somatic analogues, the histones. Histones are among the most highly

Table 1 Loss of function of semen coagulum proteins

Protein	Species	Lesion	Reference
Semenogelin 1 and 2 (SEMG1 and SEMG2)	*Gorilla gorilla* (Gorilla)	Alleles with premature stop codons	Jensen-Seaman and Li (2003); Kingan *et al*. (2003)
Semenogelin 2 (SEMG2)	*Saguinus oedipus* (Cotton-top tamarin)	long interspersed element 1 (LINE1) insertion	Lundwall and Olsson (2001)
Transglutaminase 4 (TGM4)	*Gorilla gorilla* (Gorilla)	Deletion, frameshift	Clark and Swanson (2005)
	Hylobates lar (Lar gibbon)	Premature stop codon	Clark and Swanson (2005)
Kallikrein 2 (KLK2)	*Saguinus oedipus* (Cotton-top tamarin)	Active site mutation, premature stop, altered androgen response elements	Olsson *et al*. (2004)
	Gorilla gorilla (Gorilla)	Gene not found	Clark and Swanson (2005)
	Symphalangus syndactylus, *Nomascus gabriellae*, *Hylobates lar* (3 gibbon species)	Gene not found	Clark and Swanson (2005)
	Macaca mulatta (Rhesus macaque)	Active site mutation	Clark and Swanson (2005)
Prostate-specific antigen (PSA)	*Saguinus oedipus* (Cotton-top tamarin)	Gene not found	Olsson *et al*. (2004)
	Callithrix jacchus (Common marmoset)	Protein absent from ejaculate	Valtonen-Andre *et al*. (2005)

conserved proteins in the proteome while protamines are among the fastest evolving and show adaptive evolution when comparing mammalian sequences (Rooney and Zhang, 1999). Their positive selection has continued in primates during the evolution of chimpanzees and humans (Nielsen *et al*., 2005). It is unclear why these proteins are under selection, but hypotheses include sperm competition related to how well the sperm head is packed and a selective pressure to maintain sufficient positive charge to bind DNA (Rooney *et al*., 2000). **See also**: DNA Packaging in Sperm

A study of human and chimpanzee sequences uncovered an overabundance of adaptive evolution among spermatogenesis proteins (Nielsen *et al*., 2005). Similarly, the tissue with the most pronounced overrepresentation of positive selection was the testis. Some of these proteins are USP26, C15orf2, PEPP-2, TCP11, HYAL3 and TSARG1. TSARG1 presents a unique hypothesis involving competition between germ cells because it has a role during apoptosis in sperm. A large proportion of potential gametes are eliminated through apoptosis. During this culling of cells, any particular cell with a new mutation allowing it to avoid apoptosis would be more likely to become a spermatozoon. This interesting proposal is supported by a significant overabundance of positive selection among apoptosis proteins.

Several other spermatogenesis proteins show adaptive evolution although further work is required to explain its causes. Two gene families with testis-specific expression, the *PRAME* and *SPANX* families, have expanded greatly during human evolution and show evidence of positive selection (Kouprina *et al*., 2004; Birtle *et al*., 2005; Gibbs *et al*., 2007). Their expansions could indicate new requirements in spermatogenesis in humans and our close predecessors. Likewise, the testis proteins, RSBN1 and NR0B1, underwent selective events in human populations (Voight *et al*., 2006). These recently discovered cases highlight potential novelties of spermatogenesis in our species.

Conserved Proteins

Considering the cases presented in this article, it is important to note that there are conserved sperm proteins. Proteins involved in internal signalling and basic cellular functions of sperm are largely conserved. This is illustrated well by the fact that orthologues of mammalian sperm proteins can be identified in invertebrates, such as those proteins that carry out the acrosome reaction (Vacquier, 1998). Whereas, rapidly evolving primate proteins can be difficult to identify in other mammals, and sometimes an entirely different protein has taken up its function. For example, semen coagulation proteins are different between rodents and primates.

Conclusion

Each stage of a sperm's existence, from spermatogenesis to fertilization, contains proteins under adaptive evolution. The adaptations are thought to be in response to

sexual selection, pathogens or selection against hybrids. The divergence of sperm proteins also involves constant coevolution with female proteins. It is important to be aware of these forces when studying sperm proteins, because adaptation, and hence rapid evolution, is seen at important functional regions. Extreme divergence can indicate functional importance just as extreme conservation does in other proteins. There is an overabundance of sperm proteins under positive selection relative to other functional categories, indicating their importance in the evolution of primates. However, adaptive evolution is observed in reproductive proteins from plants, green algae, diatoms, molluscs, insects, echinoderms and vertebrates, so it is likely that primate sperm proteins experience selective pressures shared by most sexual species.

Text Abbreviations

ACPP	Prostatic acid phosphatase
C15orf2	Hypothetical protein LOC23742
CAMP	Cathelicidin antimicrobial peptide
CATSPER	Sperm-associated cation channel
HYAL3	Hyaluronidase 3
MSMB	Beta-microseminoprotein
NR0B1	Nuclear receptor subfamily 0, group B, member 1
ODF2	Outer dense fiber of sperm tails 2
PEPP-2	Paired-like homeobox protein
PH20	Sperm surface protein PH-20
PKDREJ	Ploycystic kidney disease and receptor for egg jelly-related protein
RSBN1	Round spermatid basic protein 1
SED1	Secreted protein containing EGF repeats and Discoidin/F5/8 complement domains
SPAG4	Sperm associated antigen 4
SPAG6	Sperm associated antigen 6
SPAM1	Sperm-adhesion, molecule1
suREJ	Sea urchin receptor for egg jelly
TCP11	*t*-complex 11
TSARG1	*Homo sapiens* testis and spermatogenesis cell apoptosis related protein 1
USP26	Ubiquitin-specific protease 26

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