

Editorial

The small, highly basic nuclear proteins called protamine that package DNA in the sperm of all mammals, most vertebrates and many plant species were discovered nearly 150 years ago. These amazing little proteins have been found to exhibit little structural or functional similarity to any other known chromatin or DNA binding protein. The protamines, which are categorized into two closely related families, protamine P1 and protamine P2, belong to a very limited class of proteins that remain unstructured in solution and do not adopt a conformation until they bind to their intended partner or target. When these proteins are expressed inside the maturing spermatid and delivered into the nucleus, the protamines bind along the entire length of the DNA, displace the majority of the bound chromatin proteins, and globally inactivate the entire elongating spermatid genome. Working in concert with a few remaining sperm specific histones and other proteins retained in sperm chromatin, the genome is deprogrammed and selected male genes are imprinted. This reorganization prepares the sperm genome so it will no longer function as a testis cell and provides a mechanism for the reactivation of a specific subset of genes early after fertilization. Clearly this is a lot of "functionality" to attribute to any protein. However, results from recent biophysical studies suggest the protamines may also perform another function that is not typical of other DNA binding proteins. Upon binding to DNA and condensing it into toroids, the formation of the toroid generates sufficiently large forces inside the nucleus to induce the collapse of the entire spermatid genome, condense the nucleus into a very small volume and perhaps even drive the shaping of the sperm head.

The papers presented in this special HOT TOPICS issue on *Protamines: Structure and Function* review and provide new information and insight into 1) how the protamine proteins evolved and the impact of these changes on chromatin organization, 2) the processing of the protamine P2 precursor and the relationship between incomplete P2 processing and male infertility, 3) the impact of altering the proportion of the two protamines used to package DNA in the sperm cell, 4) the role endocrines play in the histone to protamine transition and chromatin condensation, 5) the forces generated by protamine when it binds to DNA, and 6) the process of protamine removal from DNA when the sperm enters the oocyte.

The Kasinsky *et al.* review, which focuses on the evolution of the protamines, shows that the arginine-rich proteins that comprise the protamine family are likely to have evolved from the lysine-rich histone H1, through a general class of protamine-like proteins to the small, highly basic protamines found in mammalian sperm. One important outcome of this change to arginine-rich protamines, as suggested in this review, is that the evolution of the protein appears to have also led to a change in the pattern of chromatin organization within the nucleus and the development of a lamellar mediated process for generating a highly condensed form of chromatin. At the molecular level, the manner in which the protamines associate with the spermatid's DNA represents an excellent example of the biological self-assembly that is initiated when the protamine molecules, which are synthesized and pumped into the nucleus of the late-stage spermatid, binds to all but a small subset of the haploid genome. Upon binding, the protein neutralizes the negative charge on the phosphodiester backbone of both strands of DNA and functions as the ultimate repressor by shutting down the transcriptional activity of the entire spermatid genome. In addition to the physical compaction of the sperm genome, which contributes directly to the suppression of its genetic activity, the formation of the complex and its compaction also appears to protect the cell's DNA from physical damage and enables the packaging of a large volume of DNA into a small, more hydrodynamic and highly specialized cell nucleus – the sperm head.

Two different types of protamine have been isolated from mammalian sperm. Protamine P1, the smaller of the two proteins, is found bound to genomic DNA in the sperm of all mammals. Protamine P2, a slightly larger protein, is found in addition to protamine P1 in the sperm of primates, rodents and a selected subset of other species. As described in the Nanassy *et al.* review, alterations in the relative proportion of the P1 and P2 protamines in human sperm have a positive correlation with male infertility. While deficiencies of either P1 or P2 have been directly linked to infertility, the cause of the infertility may be related to an indirect effect such as altered histone retention or abnormally established epigenetic marks on developmental gene promoters in the sperm chromatin that ultimately lead to abnormal early embryo development and reduced pregnancy rates.

Unlike P1, protamine P2 is synthesized as a precursor-protein that is post-translationally processed over a period of several days after the precursor binds to DNA. As described in the review by deMateo *et al.*, this processing leads to the removal of ~40% of the length of the precursor. Numerous studies have shown some P2 precursor proteins to be retained in the mature sperm of normal (fertile) human males. However, the relative proportion of unprocessed or partially processed P2 precursors appears to be increased significantly in sperm obtained from infertile patients. de Mateo *et al.* discusses this variation and how it correlates with the protamine P1/P2 ratio, DNA integrity and the assisted reproduction outcomes. They also report that within these patients, a marked cell to cell variation is observed in the presence and abundance of pre-P2 when measurements are performed in individual sperm cells.

Another paper in this issue reviews what we have learned about the role of the endocrine system on the histone to protamine transition and the impact FSH, testosterone and estradiol have on the process of sperm chromatin condensation. The timing of protamine expression and deposition in spermatid chromatin have been well documented in a number of different species, but very little is known about the mechanism of the histone to protamine transition and how external factors, such as hormones, affect this process. Earlier studies have shown that post-translational modifications of both the histones (acetylation) and protamines (phosphorylation) play important roles in this process. The Gill-Sharma *et al.* mini-review describes what we have learned about the role of the endocrines on processes that impact histone displacement, such as hyperacetylation of the histones, and the overall process of chromatin condensation. It also suggests that a fair percentage of deficiencies in sperm observed in infertile males may be induced by changes in endocrine function.

The Cree *et al.* paper reviews a very different aspect of protamine function. Studies of protamine-DNA complexes generated *in vitro* have shown that the neutralization of the phosphodiester backbone of DNA by the positively charged guanidinium groups present in each arginine residue in protamine results in the coiling and collapse of the DNA molecule into a toroidal subunits containing approximately 50,000 bp of DNA. The development of optical and magnetic traps has enabled investigators to examine the process of toroid formation in real time using individual DNA molecules in a manner that eliminates the complications normally encountered when working with bulk DNA- aggregation and precipitation. This article reviews what has been learned from single molecule studies about protamine binding to DNA and it also shows, for the first time, that the binding of protamine to DNA and the coiling of the loops of DNA into a toroid exerts a large force on the ends of constrained DNA molecules. Because the DNA stands in nuclei are attached at multiple points (MARs) to an inner nuclear matrix, the results suggest the force generated is sufficiently large to induce not only the condensation of the spermatid chromatin, but it might also contribute the forces needed for sperm head shaping.

Perhaps the one aspect of sperm chromatin organization that is understood the least is that described in the final paper by Jones *et al.*. Once the head of a mature sperm fertilizes and enters an oocyte, the protamines must be removed so the inactive male genome can be reactivated and initiate the changes that lead to the development of an embryo. In this paper, the authors review what is known about this process, describe nucleoplasmin like proteins that appear to play a role in removing protamines and re-loading the DNA with histones, and report new data showing the timing of this process for human sperm injected into hamster eggs.

While many aspects of protamine structure/conformation and the nature of their interactions with DNA have been deduced from studies of the native chromatin complex in sperm heads and synthetic DNA-protamine complexes prepared *in vitro*, we have learned comparatively little about the high resolution structure of the DNA-protamine complex. High-resolution 3-D structures of protamine or the DNA-protamine complex have never been determined because of the large size of the native complex, its insolubility, and the non-specific aggregation that occurs when DNA-protamine complexes are formed *in vitro*. The structure of the complex and the impact protamine binding has on the dynamics and functionality of DNA are critically important to our understanding of reproductive biology. These proteins not only play an important role in male fertility, but they also represent an unexplored future target for developing male-specific contraceptives. The complexes formed when protamine binds to double-stranded DNA also provide an excellent example of nanomolecular self-assembly. Protamines have already been used to successfully package exogenous genes into nano-particles that have been shown to improve the efficiency of gene uptake/transfection by cells. Understanding the structure of the DNA-protamine complex and the factors that lead to or control its formation could also be used in the future to assemble other nanoscale materials (wires or polymers) with unique functional or mechanical properties.

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