RESEARCH HIGHLIGHT

Epigenetic marks in zebrafish sperm: insights into chromatin compaction, maintenance of pluripotency, and the role of the paternal genome after fertilization

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Human sperm chromatin, and the sperm of most mammals, undergoes extensive remodeling during spermiogenesis during which 85%–95% of the histones are removed and replaced with protamines. The replacement of most histones with protamines facilitates a tighter packaging of the chromatin that is necessary for normal sperm function, and may help protect sperm DNA from damage during transport.[1] An intriguing question has been why the replacement of histones with protamines is not complete, and if the histones that remain in human sperm chromatin could have a programmatic role in regulating gene expression post-fertilization? Recently, genome-wide studies of human and mouse sperm have demonstrated that most key developmental gene promoters are uniquely packaged with nucleosomes containing bivalent marks, defined as the presence of both activating histone modifications and deactivating histone modifications at the same location, and the DNA is profoundly demethylated.[2,3] The bivalent marks and demethylation are similar to the bivalent poising of promoters of regulator genes seen in embryonic stem (ES) cells, and may have important ramifications and insights into embryonic gene expression and the establishment of totipotency in germ cells and/or the maintenance of pluripotency in ES cells.[4] In an exciting paper recently published in *Genome Research*, Wu *et al.* evaluated the epigenetic marks of chromatin from zebrafish sperm, a vertebrate species that does not have any protamines involved in sperm chromatin

packaging, and report that while interesting differences exist between zebrafish and human or mouse sperm chromatin packaging, the underlying pattern of bivalent packaging of developmental genes exists in zebrafish sperm.[5]

An initial aim of Wu *et al.* was to understand the condensation of protamine-free zebrafish sperm chromatin. Chromatin preparations from the zebrafish sperm confirmed that no protamines or transition proteins were present in the preparations. Canonical histones H2A, H2B, H3, histone variants H2AFX and H2AFV, and linker histone H1 and related variants were all identified from the chromatin preparations, and linker histone H1 was found at fourfold levels compared to fibroblasts. Linker histones facilitate development of chromatosomes that have an increase in the average nucleosome repeat length, which indeed was confirmed in the zebrafish sperm nucleosomes. Therefore, it appears that chromatin condensation is partially facilitated by an increased level of linker histone H1. Secondly, Wu *et al.* found that histone H4K16ac, which inhibits transition of 10-nm chromatin fibers into more dense 30-nm fibers, was virtually absent in the chromatin. It appears that these two mechanisms are largely responsible for the protamine-free compaction of zebrafish sperm chromatin.

As was reported previously for human sperm, the genome-wide location of histones with specific activating or repressing modifications and the status of DNA methylation were determined using chromatin immunoprecipitation and methylated DNA immunoprecipitation, respectively. The zebrafish sperm chromatin contained both 'repressive or silencing' histone modifications (H3K27me3

and H3K9me3) and 'activating or positive' histone modifications (H3K4me3, H3K4me2 and H3K14ac) in bulk levels comparable to zebrafish fibroblasts. As mentioned above, no H4K16ac was detected in the sperm chromatin. Sperm DNA was generally hypermethylated, although hypomethylation was observed in regions of developmental regulators, transcription factors and metabolism/biosynthesis loci.

Similar to the human, the zebrafish chromatin appears to contain activating histone modifications (H3K4me3, H3K4me2 and H3K14ac) at sets of genes that may reflect a historical record of gene activation during spermatogenesis. The spermatogenesis-related genes include genes involved in processes such as flagella/microtubule development, DNA repair and meiosis-related genes. The repressive mark H3K27me3 was seen mostly in developmentally important gene regions at high density. The activating marks H3K4me3 and H3K4me2 were also seen in more moderate levels at the same groups of developmental loci.

Importantly, the large sections of 'multivalent' or 'bivalent' chromatin described above were seen at key developmental transcription factors involved in embryonic development and similar to bivalency observed in ES cells. Similar to the human and mouse, the large bivalent and multivalent loci were hypomethylated. Human and mouse sperm display a 'bivalent poising' of H3K4me3/H3K27me3 with concurrent hyomethylation near the transcription start sites of key developmental genes. Interesting, Wu *et al.* found that the bivalency was present in larger blocks of DNA in zebrafish sperm, up to tens of kilobases at clustered gene loci and often extending throughout the entire locus.

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The presence of 'bivalently poised' nucleosomes and DNA hypomethylation at key embryonic developmental loci in a manner similar to the bivalency of such genes in ES cells suggests at least two hypotheses. First, the bivalent marks may suggest a mechanism for establishment of totipotency in the germline and maintenance of pluripotency through embryogenesis into the presumptive ES cells. In such a scenario, Wu *et al.* hypothesize that the repressive marks are necessary to preclude expression of key regulatory genes in the germline, but that positive marks are simultaneously need to avoid DNA methylation that may carryover to a repressive state in the embryo when expression is required.

The second hypothesis is that the bivalent poising may be programmatic to embryo development. In support of this possible function, Wu *et al.* note that the genes with the highest levels of activating marks are generally expressed in the zebrafish blastula before the midblastula transition, the point at which general zygotic transcription is initiated. These genes include factors that promote the cell cycle and factors involved in RNA translation and metabolism, key systems to 'prime' before the initiation of zygotic transcription. On the other hand, genes with less enrichment of activating marks

are generally transcribed later in embryogenesis. This hypothesis has obviously important implications for the role of the potential paternal contribution to normal embryogenesis beyond the current paradigm, but suggested by some other studies including outcome data from *in vitro* fertilization patients and epidemiological studies.

Ultimately, the role of sperm epigenetic marks will be better understood when similar studies are completed in oocytes and embryos. Additionally, a better understanding of the post-fertilization changes is needed in order to better understand the role of sperm epigenetic marks in embryogenesis. For example, it is well understood that a general wave of demethylation occurs following fertilization, but that imprinted genes and some other genes are unaffected.[6] Also, protamines are clearly replaced with maternal histones at the pronuclear stage.[7] At this point, it is unclear how the marks established in mature sperm may be affected by these transitions, or how they may affect the transition itself.

In conclusion, Wu *et al.* describe a unique epigenetic profile previously reported in mammalian sperm, but now confirmed in a fish species that is evolutionary removed from humans, and does not employ protamines to accomplish sperm chromatin condensation. The epigenetic profile includes an apparent

historical record of genes activated during spermatogenesis, as well as a unique bivalent poising of key developmental genes of embryogenesis. The bivalent poising of developmental genes and the degree of activating mark enrichment may be instructive and temporally functional during embryonic gene activation, and/or may be important in understanding how totipotency is established in the germ cells and pluripotency maintained in the embryos.

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