Title (Capitalize the first letter only)

Re-evaluating the localization of sperm-retained histones revealed the modification-dependent accumulation in specific genome regions

Authors (*Corresponding author)

Kosuke Yamaguchi, Masashi Hada, *Yuki Okada

Affiliations

Institute for Quantitative Biosciences, the University of Tokyo

E-mail

ytokada@iam.u-tokyo.ac.jp

Abstract (300 words maximum in Font 10)

In testis, marked chromatin condensation occurs during spermiogenesis, as the majority of histones are replaced by Transition Proteins and subsequently by Protamines. During the nuclear elongating period, incorporation of histone variants and specific histone modifications affect the higher-order chromatin structure and play critical roles in chromatin compaction, which appears to be critical for the maintenance of genome integrity. By the LC-MS screening of sperm histones, we successfully identified a novel phosphorylation on TH2A, a germ cell-specific histone H2A variant at its 127th Threonine residue (= TH2A-pThr127). This amino acid residue is unique to the TH2A and phosphorylated concomitant with chromatin condensation in both spermiogenesis and meiotic pericentromere by Haspin, suggesting its involvement in chromatin compaction, although our knockout mouse study indicated that it's functionally dispensable.

Simultaneously, we also faced contradictory observations regarding the localization of TH2A-pThr127 in sperm genome; whether it localizes to gene coding regions or gene deserts. In fact, similar issue has been debated for years, and it's likely caused by the non-uniform sensitivity of sperm chromatin to micrococcal nuclease (MNase) digestion. In order to overcome this issue, we utilized nucleoplasmin (NPM) to improve the solubility of sperm chromatin by removing Protamines in vitro. NPM treatment efficiently solubilized histones while maintaining quality and quantity. Chromatin Immunoprecipitation-sequencing analyses using NPM-treated sperm demonstrated the predominant localization of H4 to distal intergenic regions, while modified histones exhibited a modification-dependent preferential enrichment in specific genomic elements, such as H3K4me3 at CpG-rich promoters and H3K9me3 in satellite repeats, respectively, implying the existence of protection machinery of modified histones from eviction.

Key words (Not more than five)

Sperm-retained histones, TH2A, Haspin, ChIP-seq, Nucleoplasmin,