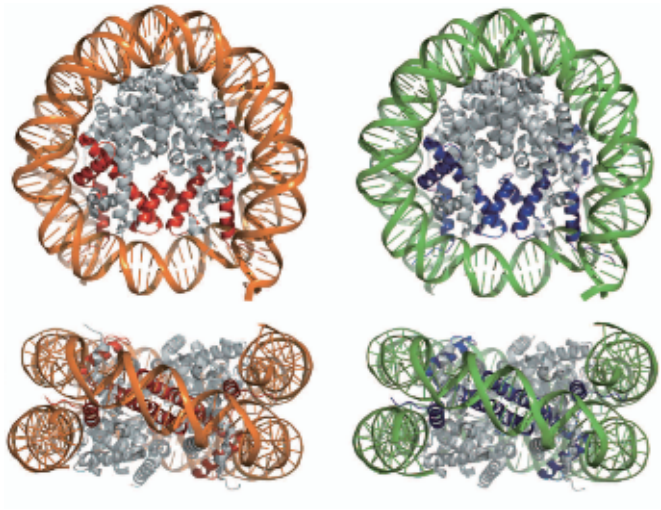
Crystal structure of the human nucleosome containing a testis-specific histone variant, H3T

Genomic DNA is compacted into the nucleus in the form of chromatin, which possesses the fundamental repeating structure called the nucleosome. In the nucleosome, a 146 base-pair DNA is wrapped 1.7 times around the histone octamer, which contains two each of the core histones H2A, H2B, H3, and H4. These core histones are composed of flexible N- and/or C-terminal tails and the histone-fold domain. The histone-fold domain provides interaction surfaces between the histones H2A and H2B (H2A/H2B), and between the histones H3 and H4 (H3/H4).

Nonallelic isoforms of histones have been identified in higher eukaryotes. For the histone H3, seven variants, i.e., H3.1, H3.2, H3.3, H3T, H3.X, H3.Y, and CENP-A, have been reported, and their common and specific functions have been discussed. H3.1 and H3.2 are expressed at the S phase of the cell cycle, and incorporated into chromatin in a replication-dependent manner. By contrast, H3.3 is constitutively expressed, and is incorporated into transcriptionally active chromatin regions and telomeres in a replication-independent manner. CENP-A is a centromere-specific H3 variant. The histones H3.X and H3.Y are novel histone variants that may be involved in the regulation of cellular responses to outside stimuli. The histone H3T was found as the testis-specific H3 variant in mammals. It may play an important role in the chromatin reorganization required for spermatogenesis.

We previously reported that H3T can be assembled into nucleosomes with H2A, H2B, and H4 (H3T nucleosome) [1]. The H3T nucleosome is not assembled by the conventional histone chaperone Nap1 [1,2]. On the other hand, Nap2 efficiently promotes the assembly of the H3T nucleosome [1,2]. These findings suggest that the H3T nucleosome has a specific structural feature and is assembled into chromatin by a specific chaperone-mediated pathway in the testis. H3T was also found in the comprehensive proteome analyses of nuclear extracts from HeLa cells. However, the nucleosomes containing H3T probably comprise an extremely small proportion of bulk chromatin in somatic cells. Therefore, H3T may have a limited function in somatic cells that is currently unknown.

To study the structure and function of the H3T nucleosome, we first prepared the human histones H2A, H2B, H3T, and H4, as bacterially expressed recombinant proteins [3]. The H3T nucleosome containing H2A, H2B, H3T, H4 and 146-base-pair DNA was reconstituted by the salt dialysis method and purified using the Prepcell apparatus. Our biochemical and cell biological experiments revealed that the H3T nucleosome is extremely unstable as compared with the H3.1 nucleosome, *in vitro* and *in vivo* [3]. To study the structural basis of the instability of the H3T nucleosome, the H3T nucleosome was crystallized and its crystal structure was determined at a 2.7 Å resolution (Fig. 1) [3]. We also crystallized the nucleosome containing the conventional histone H3.1, instead of H3T, and the crystal structure of the H3.1 nucleosome was determined at a 2.5 Å resolution (Fig. 1). The H3T nucleosome and H3.1 nucleosome crystals belonged



H3T-nucleosome **H3.1-nucleosome**

Fig. 1. Crystal structures of the H3T and H3.1 nucleosomes.

to the orthorhombic space group $P2_12_12_1$, with similar unit cell constants (a=105.5 Å, b=109.5 Å, c=181.1 Å for the H3T nucleosome, and a=105.8 Å, b=109.5 Å, c=180.9 Å for the H3.1 nucleosome). In both crystals, one nucleosome was present in the asymmetric unit. Diffraction data were obtained using beamline **BL41XU**. The structures of the H3T and H3.1 nucleosomes were determined by the molecular replacement method, using the previously solved human nucleosome structure (PDB ID: 2CV5) as a search model [4].

The overall structure of the H3T nucleosome was essentially similar to that of the H3.1 nucleosome (Fig. 1). However, careful structural comparison between H3T and H3.1 nucleosomes revealed significant differences between H3T and H3.1 near both ends of the central α2 helix (Fig. 2). At these distorted sites, the van der Waals radii of the Met71 and Val111 residues come in close contact with that of the Val89 and Asp123 residues, respectively. The Met71 and Val111 residues are H3T-specific residues (in H3.1, Val71 and Ala111). Our mutational analysis revealed that the Met71 and Val111 residues contributed to the instability of the H3T nucleosome [3].

In the present study, we found that the H3T nucleosome structurally and biochemically differs from the conventional H3.1 nucleosome. H3T is highly expressed in the testis, and is anticipated to have a specific function in chromatin reorganization during sperminogenesis, in which drastic chromatin reorganization occurs by histone replacement with histone variants and protamines. The unstable nature of the H3T nucleosome may promote this global transition of the chromosome architecture during meiotic and/or postmeiotic events. It has been reported that about 4% of the sperm haploid genome is retained in nucleosomes [5]. The H3T nucleosomes may also be a constituent of sperm chromatin. Interestingly, sperm nucleosomes are significantly enriched around developmentally important genes, such as imprinted gene clusters, microRNA clusters, and *HOX* gene clusters, suggesting that these sperm nucleosomes have a specific epigenetic function. The specific biochemical and structural properties of the H3T nucleosome found in the present study may play an important epigenetic role in sperm chromatin.

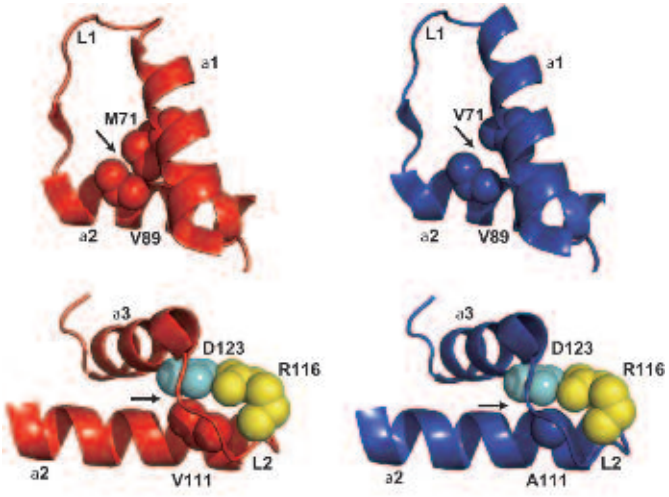


Fig. 2. Close-up views of the H3T region structurally different from H3.1. Arrows indicate the locations of H3T and H3.1 that are structurally different from each other.

Hiroaki Tachiwana, Wataru Kagawa and Hitoshi Kurumizaka*

Laboratory of Structural Biology, Waseda University

*E-mail: kurumizaka@waseda.jp

References

[1] H. Tachiwana *et al.*: Nucleic Acids Res. **36** (2008) 2208.
[2] *H. Tachiwana, W. Kagawa, A. Osakabe, K. Kawaguchi, T. Shiga, Y. Hayashi-Takanaka, H. Kimura and H. Kurumizaka: Proc. Natl. Acad. Sci. USA **107** (2010) 10454.*
[3] A. Osakabe *et al.*: J. Biol. Chem. **285** (2010) 11913.
[4] Y. Tsunaka *et al.*: Nucleic Acids Res. **33** (2005) 3424.
[5] S.S. Hammoud *et al.*: Nature **460** (2009) 473.