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A K52Q substitution in the globular domain of histone H1t modulates its nucleosome binding properties

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Abstract **A comparison of the globular domain sequences of the somatic H1d and testis-specific H1t revealed a single substitution of lysine 52 in H1d to glutamine 54 in H1t, which is one of the three crucial residues within the second DNA binding site. The globular domains of both histones were modeled using the crystal structure of chicken GH5 as a template and was also docked onto the nucleosome structure. The glutamine residue in histone H1t forms a hydrogen bond with main chain carbonyl of methionine-52 (in H1t) and is spatially oriented away from the nucleosome dyad axis. A consequence of this change was a lower affinity of recombinant histone H1t towards Four-way junction DNA and reconstituted 5S mononucleosomes. When Gln-54 in Histone H1t was mutated to lysine, its binding affinity towards DNA substrates was comparable to that of histone H1d. The differential binding of histones H1d and H1t towards reconstituted mononucleosomes was also reflected in the chromatosome-stop assay.**

Keywords: Histone H1t; Globular domain; Nucleosome binding property

1. Introduction

The linker histone H1 has been implicated in the formation and stabilization of higher order chromatin structure in the eukaryotic nucleus [1]. In mammals ten variants, including the recently discovered oocyte specific histone H1oo and the spermatid specific histone H1s, H1T2 and H1LS, have been described [2–7]. H1T2 is expressed during steps 9–14 of spermiogenesis and is essential for spermatid elongation and DNA condensation [4,5]. H1LS gene is expressed in step 4 spermatids peaking around steps 6–8 spermatids. H1LSp is implicated in the chromatin remodeling process during the final stages of spermiogenesis [6,7].

The histone variants have conserved amino acid sequences in the globular domain but differ considerably in their sequences at the C-terminus. Histone H1t, whose expression starts at the mid-pachytene interval, is a poor condenser of DNA and chromatin in vitro [8,9] due to the absence of an octapeptide repeat motif containing the DNA binding SPKK units in its C-terminus adapting an HMG-box domain like structure [10,11]. The same stretch was shown to be responsible for in vitro DNA condensation [12] and stabilization of chromatin structure [13].

The three dimensional structure of the globular domain is now available based on the X-ray crystal structure of GH5 [14] and NMR structure of GH1 [15]. The globular domain binds to nucleosome through two binding sites. The primary DNA binding site comprises of residues Lys 69, Arg 73 and Lys 85 that are present in helix III. A cluster of highly conserved basic residues (Lys 40, Arg 42, Lys 52 and Arg 94) on the opposite side of the globular domain, about 25–30 Å away from the primary binding site, was proposed to constitute a less defined secondary DNA binding site. Both the proposed DNA binding sites were shown to be required for GH5 binding to nucleosome [16]. The four residues within the secondary DNA binding domain function as a cluster but are individually dispensable for binding to DNA and nucleosomal templates [17]. Recently, we have modeled the entire chromatosome particle [18]. In the present study, we have built an atomic model of histone H1t globular domain based on the crystal structure of GH5 and find that the second DNA binding site exhibits significant differences from that of histone H1d. We have carried out a detailed comparative analysis of its in vitro binding properties to Four-way junction DNA and mononucleosomes with reference to somatic histone H1d.

2. Materials and methods

2.1. Molecular modeling of the globular domains of histones H1d and H1t and positioning of the globular domains with respect to nucleosome

The amino acid sequences of rat H1d (Acc. No. P15865) and rat H1t (Acc. No. P06349) were obtained from the SWISSPROT database. Sequence alignments and analysis were carried out using the GCG package [19]. CLUSTALW was used to carry out multiple sequence alignments [20]. Secondary structure predictions were carried out using the neural network based Predictprotein algorithm [21]. The three dimensional structures of the globular domain in H1d and H1t were built using the homology modeling technique with the help of the MSI software package (Insight II, Accelerys Inc.), based on the crystal structure of the globular domain of chicken histone H5 (GH5, PDB 1HST). Structure manipulations, docking and analysis were also carried out using the same package. Side chains that differed from the template were initially placed according to their rotamer preferences

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as per the Ponder and Richard's rotamer library. Wherever this resulted in severe steric clashes, they were manually adjusted to the next preferred combination. A 5 Å shell of water molecules was added to surround the protein molecule and the structure was subjected to energy minimization using a 13 Å nonbonded cutoff and a distance dependent dielectric constant, initially using the steepest descent algorithm followed by the conjugate gradient method till the root mean square derivative was less than 0.4 kcal mol A. The globular domain of H1t was then docked onto the molecular model of the chromatin particle built by us recently [18]. A structural superposition of GH1t with GH1d enabled to position GH1t on the nucleosome.

2.2. Cloning and expression of rat histone H1t in Escherichia coli and its purification

The plasmid containing the histone H1t genomic locus was a generous gift from Dr. W.S. Kistler (Columbia, South Carolina, USA). The coding sequence of histone H1t was amplified and cloned into pTrc99A expression vector using *E. coli* DH5α. Although no visible overexpressed band was observed upon induction with IPTG, the recombinant histone H1t protein was purified as described for histone H1d [22] except that the binding to the Ni^{2+}-agarose bound fraction on heparin agarose was done at 0.4 M NaCl instead of 0.5 M NaCl. The authenticity of recombinant histone H1t was confirmed by N-terminal sequencing.

2.3. Generation of globular domain mutants of histones H1d and H1t

Site-directed mutants of the globular domain of both histone H1d and H1t proteins were created using the modified mega primer method [23]. The primer containing the desired mutation (3′ primer) and the gene specific 5′ primer was used to generate a fragment of the gene bearing the desired mutation. The primers used to generate H1dK52Q and H1tQ54K mutants were 5′-GCT GCG CTC CTG GGA GGC GGC-3′ and 5′-TGC CCG TTC CTT AGA CAT AGG-3′, respectively.

2.4. Gel retardation assay of Four-way junction DNA with recombinant H1t and H1d

Four oligonucleotides (49-mer each) having partial segments of complementarity were used to generate labeled Four-way junction [22]. For gel retardation assay, increasing amounts of both recombinant H1d and H1t proteins were pre-incubated in 150 mM NaCl for 30 min at 4 °C. The protein solution was subsequently diluted to 30 mM NaCl with GRA buffer (10 mM Tris–HCl, pH 7.5 and 5% glycerol). Sonicated salmon sperm DNA was used as a competitor at a final concentration of 50 μg/ml. Radioactive Four-way junction DNA (15 ng, 15000 cpm) was added and binding was allowed to proceed at 22 °C for 45 min. Unbound Four-way junction DNA and H1-bound complexes were separated on a 6% native PAGE using Tris–glycine as running buffer (50 mM Tris–HCl pH 7.5 and 50 mM glycine). The gel was dried and subjected to autoradiography. The band intensities were quantified using ImageGuage software version 3.0 of Fuji Phosphorimager and the binding constants were calculated using the Scatchard equation $\{K_a = v/L(1 - v)$ where, v is the fraction bound and L is the concentration of protein that was bound$\}$.

2.5. Nucleosome reconstitution and binding of H1 proteins to mononucleosomes

Donor chromatin and nucleosome core particle were prepared from rat liver nuclei. To remove all the histone H1 proteins in the core particle preparation, the mononucleosome preparation was treated with CM-sephadex (12 mg of CM-sephadex for every 20 A_{260} units) in 0.35 M NaCl. The monomeric 5S DNA used for reconstitution (200 bp) was labeled by radioactive PCR from the plasmid pTZ-1-5S (containing a single copy of 5S rDNA from *Xenopus borealis*, a gift from Dr. U.K. Laemmli) using M13 forward and reverse primers. The mononucleosome on the radioactive 5S DNA was reconstituted by the method described by Nightingale et al. [24]. Increasing amounts of recombinant histone H1 proteins were incubated with a fixed amount of reconstituted mononucleosomes in a buffer containing 10 mM Tris–HCl, pH 7.4/25 mM NaCl/1 mM EDTA/5% glycerol at 22 °C for 30 min. The resulting complex was resolved on a 0.8% agarose gel with TBE as running buffer.

2.6. Chromatosome-stop assay

The 5S DNA template used for reconstitution (200 bp) was labeled at a single site as mentioned by Ura et al. [25]. The 5S DNA was cut at 131st position by *Sau 3A1* enzyme. The resulting fragments were dephosphorylated by calf intestinal phosphatase and subsequently labeled with γ-^{32}P-ATP. The fragments were re-ligated and digested with *Sac*I and *Bam*H1 to remove the end labeled phosphates. This single-site internally labeled fragment was reconstituted into nucleosomes as mentioned previously. Histone H1 protein was allowed to bind to reconstituted mononucleosomes as mentioned earlier, the only difference being, EDTA was omitted in the binding buffer. Subsequently, $CaCl_2$ was added to a final concentration of 5 mM and 1 μg of non-specific DNA was also added. Increasing amounts of Mnase (SIGMA) was added and the reaction was allowed to proceed for 1 min. Alternatively, 0.002 U of Mnase was used for digestion and the reaction terminated at different time points. The reaction was terminated using stop buffer containing 0.5% SDS, 5 mM EDTA and 0.2 μg/μl proteinase K and incubated for 3 h at 56 °C. The DNA fragments were resolved on a 8% native PAGE gel using TBE as running buffer. The gel was dried and subjected to autoradiography. The band intensities were calculated as mentioned earlier.

3. Results and discussion

The amino acid sequences of rat histone H1t and H1d are given in Fig. 1A. The histone H1t sequence was found to be 60% similar to that of histone H1d over its entire length of 208 amino acid residues. The amino acid sequences of the globular domains histone H1t from rat, mouse, human, pig and monkey are given in Fig. 1B along with histone H1d and chicken histone H5. While the primary binding site residues were conserved in all the histone H1 species, one of the secondary binding sites harbored a glutamine instead of a lysine (Fig. 1B) in all H1ts, suggesting it to be an H1t specific change.

3.1. Modeling of the globular domain of histone H1t

In order to assess whether the observed lysine-to-glutamine change in rat histone H1t could influence the structure and hence binding of its globular domain to nucleosome, molecular models of both the histones were built using the crystal structure of the globular domain of histone H5 as template. The globular domains of rat histone H1d and rat histone H1t shared considerable sequence homologies of 55% and 51%, respectively with the globular domain of chicken histone H5. The atomic models built by comparative modeling methods were as expected, very similar to that of GH5 in their overall features (Fig. 2A). The domain consists of a three-helical bundle with a β-hairpin at the carboxy-terminus, which is anchored to the base of the helical core. A helix-turn-helix motif containing two lysines provided a primary recognition site for DNA binding in both the structures (lysines 81, 85 in histone H1d; lysines 83, 87 in H1t corresponding to lysines 69 and 73 in histone H5). Although the overall structure of GH1d and GH1t are very similar, a significant difference between the two structures was noted. Lysine 52 is highly conserved in all somatic histone H1s of mouse, rat and human and is a part of the second DNA binding site. The testis specific histone H1t, however, has a glutamine residue at this position, which forms a hydrogen bond with the main chain carbonyl of Met 52 (in H1t) in the energy minimized structure, thus pointing away from the possible nucleic acid binding position unlike in GH5 and GH1d.

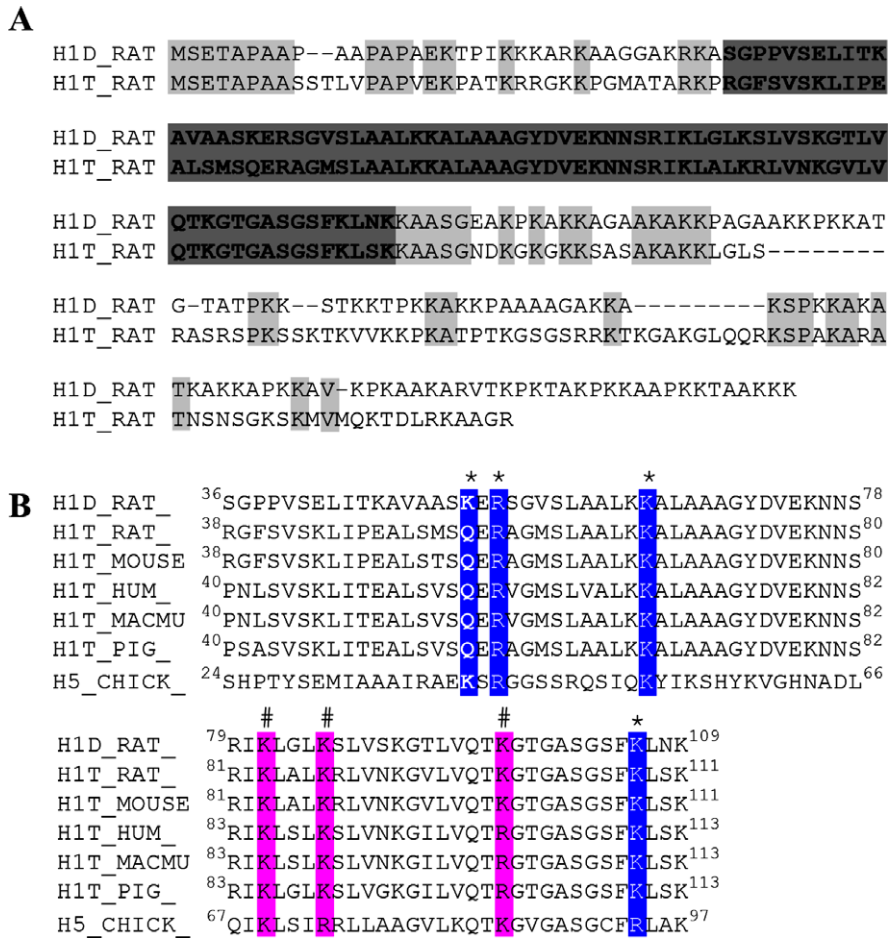

Fig. 1. Sequence analysis of histone H1t. (A) A comparison of the amino acid sequences of somatic histone H1d and histone H1t. Residues of the globular domain are enclosed in a box. Identical amino acids in the two are highlighted in gray. (B) A comparison of the globular domains of all known histone H1ts with that of histone H1d and chicken H5. Residues involved in the primary DNA binding site are depicted by a shaded pink box and a hash and residues involved in the proposed secondary DNA binding site by a shaded blue box and an asterix.

An overall high similarity between the GH1d and GH1t structures made it possible to utilize the same coordinates to position the globular domain of histone H1t on the nucleosome core as was described for histone H1d [18, Fig. 2B]. It can be seen that the primary binding sites of the globular domain are positioned to interact with the major groove of the DNA helix while, the amino acids of the second DNA binding site interact with the minor groove of the DNA helix in the dyad axis. It is evident from this model that Gln 54 present in histone H1t is oriented differently from the Lys 52 of histone H1d and is not in a position to interact with the DNA of the dyad axis of the nucleosome core particle. In addition to this major change in the structure of the globular domain of histone H1t, there are other minor changes in the distribution of basic amino acid residues. For example, the introduction of an arginine residue at position 88 (H1t) in place of a serine at position 86 (H1d), adjacent to the primary DNA binding site necessitates the rotation of histone H1t globular domain with respect to histone H1d in order to accommodate the extra length of the side chain. However, we would like to point out here that this change from serine to arginine is observed only in rat and mouse and not in pig, monkey or humans (Fig. 1B). Hence, it would be difficult to comment at present, regarding the significance of this replacement. The other two residues suggested to be part of the second DNA binding site, are similar in both H1d and H1t sequences and structures. Of these, Lysine 66 (corresponding to 52 in histone H5 and lysine 64 in histone H1d, respectively) is oriented appropriately to make a water-mediated interaction with the nucleosome, whereas Lys 108 (corresponding to Arg 94 in H5) is located too far away from either of the binding sites and hence is unlikely to influence binding of the globular domain to the nucleosomes.

3.2. Binding of histone H1d and H1t to Four-way junction and nucleosome

Since our modeling exercise showed that the secondary DNA binding site that interacts with the nucleosome core dyad axis is altered in histone H1t, we were curious to compare the binding properties of histones H1d and H1t towards both Four-way junction DNA and reconstituted mononucleosomes. For this purpose, rat histone H1t was expressed in *E. coli* (Fig. 3A). As seen in lane 3, recombinant histone H1t could be purified to near homogeneity and it migrated just above the position of native histone H1t. A slightly slower mobility of the recombinant histone H1t in the gel is due to hexa-histidine

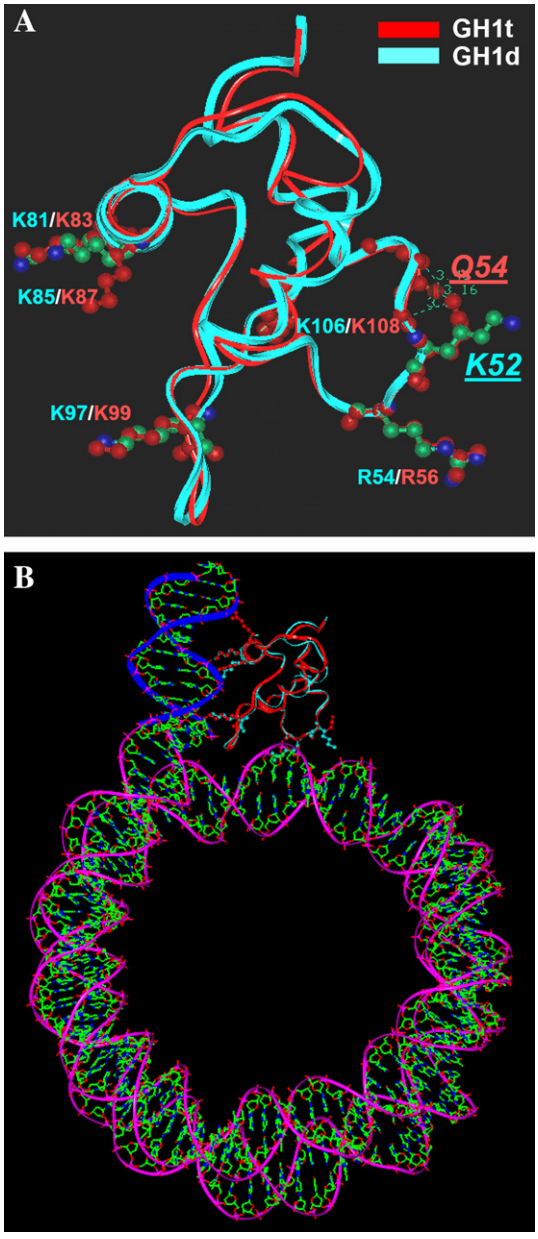

Fig. 2. Modeling of the globular domain of histone H1t. (A) A superimposition of the modeled globular domains of H1d and H1t. Histone H1t chain is depicted in red while that of H1d in cyan. Glutamine 54 of histone H1t has been shown to fold back and form a hydrogen bond with the backbone of methionine-52. (B) The same superposition shown in the context of nucleosome core particle, illustrating the location of the globular domains of histones H1d and H1t molecules (shown in cyan and red ribbons, respectively).

tag at its C-terminus. We have used recombinant somatic histone H1d [22] as a somatic counterpart in our study. The results of binding experiments with Four-way junction are shown in Fig. 3B. If the DNA–protein complexes are compared across panels in Fig. 3B, it is obvious that the complex starts appearing at a concentration of 50 ng in the case of histone H1d while in the case of H1t it appears at a protein concentration of 100 ng suggesting that there indeed is a difference in the affinity of the two proteins towards Four-way junction DNA. A quantitative picture of the differences in the binding efficiencies of the two proteins is given in Fig. 3D. The affinity constant, K_a, was calculated to be $0.14 \pm 0.02 \times 10^8\ M^{-1}$ for histone H1d and $0.07 \pm 0.01 \times 10^8\ M^{-1}$ for histone H1t. Binding experiments were carried out with reconstituted mononucleosomes also, the results of which are shown in Fig. 3C and E. The binding constant, K_a, was calculated to be $1.32 \pm 0.02 \times 10^8\ M^{-1}$ for histone H1d and $0.73 \pm 0.02 \times 10^8\ M^{-1}$ for histone H1t. In order to provide conclusive evidence that the differences in the binding affinity is indeed due to the single amino acid substitutions, we also generated the reverse mutants of both H1d and H1t (Fig. 4E) wherein the crucial Lys 52 in H1d was changed to glutamine (H1dK52Q) and the corresponding Gln 54 in H1t was changed to lysine (H1tQ54K). The mutant H1dK52Q displayed a lower binding affinity towards Four-way junction and had an affinity value that was similar to that of histone H1t ($K_a = 0.07 \pm 0.01 \times 10^8\ M^{-1}$ Fig. 4A and C). Conversely, the H1t mutant, H1tQ54K, showed a higher affinity ($K_a = 0.12 \pm 0.01 \times 10^8\ M^{-1}$,) towards Four-way junction. As found in the case with Four-way junction, the mutants displayed expected altered binding affinity with the reconstituted nucleosomes also (Fig. 4B and D). The mutant H1dK52Q had a binding affinity of $0.69 \pm 0.02 \times 10^8\ M^{-1}$, a value similar to that of histone H1t, while the mutant H1tQ54K had a binding affinity value ($1.25 \pm 0.05 \times 10^8\ M^{-1}$) that was similar to that of histone H1d. Thus, it appears that histone H1t has at least 2 fold lesser affinity to both mononucleosomes and Four-way junction DNA, than that of histone H1d. The circular dichroic spectra of the wild type and mutant histone proteins showed that there are no gross alterations in their secondary structures (data not shown).

The chromatosome stop assay has been used as a functional assay for correct histone H1 binding [26]. The reconstituted mononucleosomes, with fixed ratios of histone H1 to DNA, was checked for protection against micrococcal nuclease digestion. The digestion was performed both as a function of time (Fig. 5A and C) and as a function of Mnase concentration (Fig. 5B and D). Without histone H1, no 166 bp chromatosome band was observed (–H1 panel in Fig. 5A and B). When the histone H1d bound chromatosome was digested with Mnase, the pause at the 166 bp chromatosome level is very evident. The protection offered by histone H1d at the 166 bp band continues up to 1 min of digestion with 0.002 U of Mnase (Fig. 5A). Histone H1t bound chromatosome also gives the same chromatosome band indicating that it binds to nucleosomes just as other H1 histones. But when we compared the chromatosome band protected by histones H1d and H1t, we found that histone H1t offers lesser protection against Mnase digestion. As expected, the mutant histone proteins H1dK52Q and H1tQ54K also gave the chromatosome band but the extent of protection was different; histone H1dK52Q exhibited protection pattern that was similar to histone H1t while histone H1tQ54K paused at the chromatosome level for a longer time. The band intensities of the 166 bp fragments were quantitated and graphically represented in Fig. 5C. Similar chromatosome stop assay performed as a function of Mnase concentration also yielded similar band protection patterns (Fig. 5B). Histone H1d bound chromatosome offered protection against Mnase digestion up to 0.006 U of the enzyme, while the band disappeared at 0.004 U itself in case of histone H1t bound chromatosome. Conversely, Histone H1dK52Q offered lesser protection when compared to histone

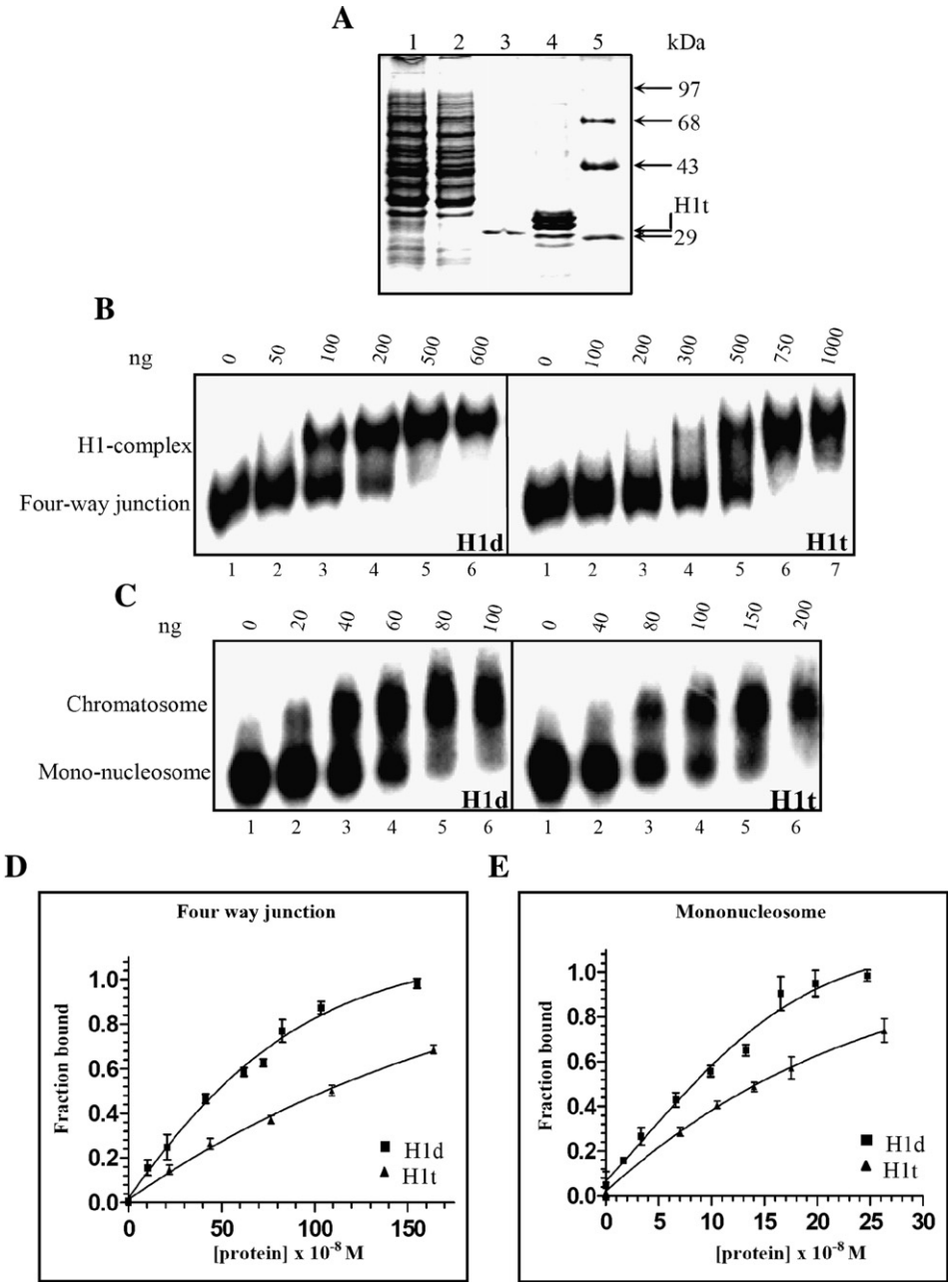

Fig. 3. Comparison of binding properties of histones H1d and H1t. (A) SDS gel profile of overexpressed and purified histone H1t protein. Lane 1: Uninduced *E. coli* BL21(DE3) total cell extract. Lane 2: Induced *E. coli* BL21(DE3) total cell extract. Lane 3: Purified histone H1t protein. Lane 4: Testis acid extract showing histone H1s. Lane 5: Molecular weight marker. (B) Binding of histones H1d and H1t to Four-way junction DNA. Lane 1 in all panels corresponds to free probe. Lanes 2–6 in H1d panel correspond to protein amounts of 50 ng (8×10^{-8} M), 100 ng (17×10^{-8} M), 200 ng (33×10^{-8} M), 500 ng (83×10^{-8} M) and 600 ng (100×10^{-8} M), respectively. Lanes 2–7 in H1t panel correspond to protein amounts of 100 ng (17×10^{-8} M), 200 ng (33×10^{-8} M), 300 ng (50×10^{-8} M), 500 ng (83×10^{-8} M), 750 ng (125×10^{-8} M) and 1000 ng (166×10^{-8} M), respectively. (C) Binding of histones H1d and H1t to reconstituted mononucleosomes. Lanes 2–6 in H1d panel correspond to protein amounts of 20 ng (3×10^{-8} M), 40 ng (6.5×10^{-8} M), 60 ng (10×10^{-8} M), 80 ng (13×10^{-8} M) and 100 ng (17×10^{-8} M) of protein, respectively. Lanes 2–6 in H1t panel correspond to protein amounts of 40 ng (6.5×10^{-8} M), 80 ng (13×10^{-8} M), 100 ng (17×10^{-8} M), 150 ng (25×10^{-8} M) and 200 ng (33×10^{-8} M), respectively. (D) Binding curve of histones H1d and H1t to Four-way junction DNA. (E) Binding curve of histones H1d and H1t to reconstituted mononucleosomes. The points in the graph represent the mean of three independent experiments and the error bars correspond to the standard deviation.

H1tQ54K. The band intensities are again graphically represented in Fig. 5D.

The affinity constants that we have observed with histone H1s to mononucleosomes are in the same order of magnitude as that observed by Talasz et al. [27] who have studied the binding properties of histone variants that were purified from mouse liver. In the present study we have observed that the protection offered by histone H1t is lesser than that offered by histone H1d. Although there is considerable sequence divergence of the C-terminus between histone H1d and histone H1t,

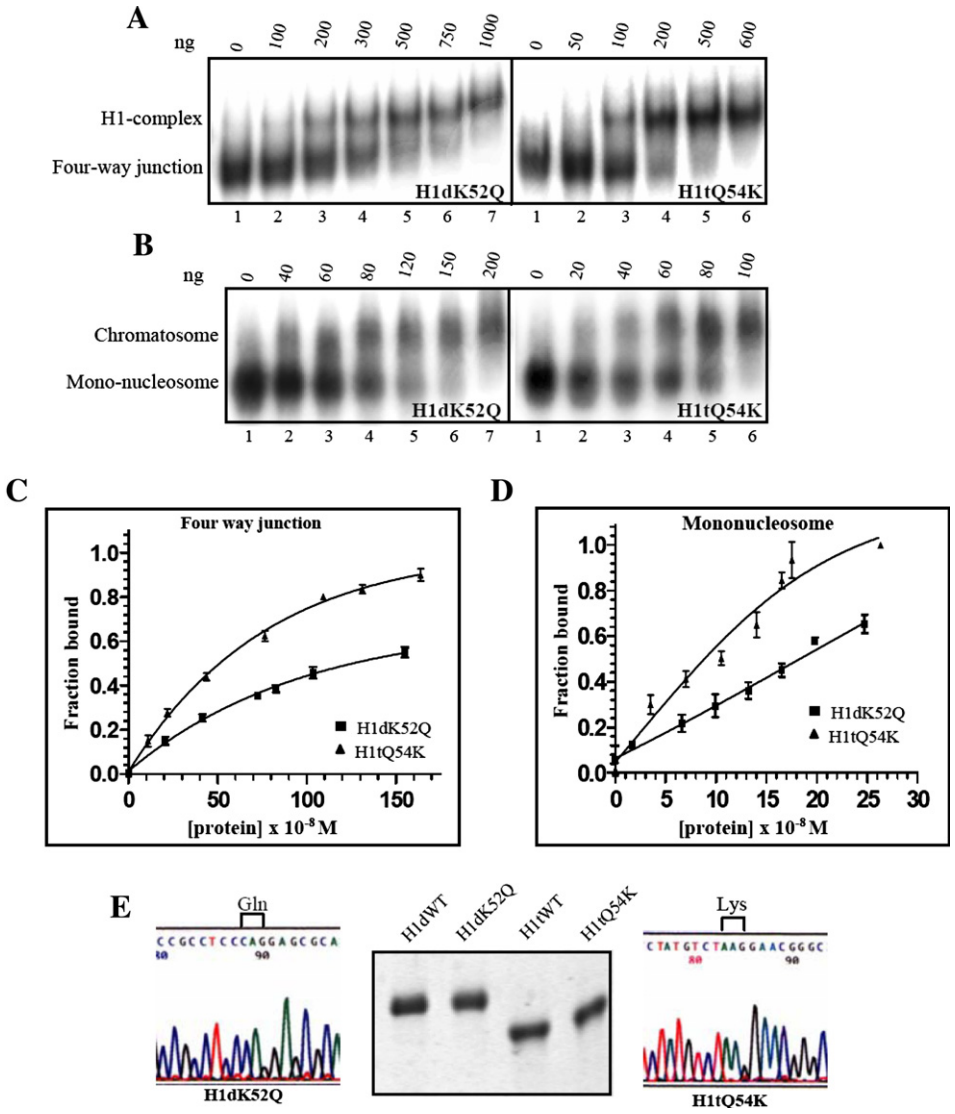

Fig. 4. Comparison of binding properties of the globular domain mutants H1dK52Q and H1tQ54K. (A) Binding of histones H1dK52Q and H1tQ54K to Four-way junction DNA. Lane 1 in all panels corresponds to free probe. Lanes 2–7 in H1dK52Q panel correspond to protein amounts of 100 ng (17×10^{-8} M), 200 ng (33×10^{-8} M), 300 ng (50×10^{-8} M), 500 ng (83×10^{-8} M), 750 ng (125×10^{-8} M) and 1000 ng (166×10^{-8} M), respectively. Lanes 2–7 in H1tQ54K panel correspond to protein amounts of 50 ng (8×10^{-8} M), 100 ng (17×10^{-8} M), 200 ng (33×10^{-8} M), 500 ng (83×10^{-8} M), and 600 ng (100×10^{-8} M), respectively. (B) Binding of histones H1d and H1t to reconstituted mononucleosomes. Lanes 2–7 in H1dK52Q panel correspond to protein amounts of 40 ng (6.5×10^{-8} M), 60 ng (10×10^{-8} M), 80 ng (13×10^{-8} M), 120 ng (20×10^{-8} M), 150 ng (25×10^{-8} M) and 200 ng (33×10^{-8} M) of protein, respectively. Lanes 2–6 in H1tQ54K panel corresponds to protein amounts of 20 ng (3×10^{-8} M), 40 ng (6.6×10^{-8} M), 60 ng (10×10^{-8} M), 80 ng (13×10^{-8} M) and 100 ng (17×10^{-8} M), respectively. (C) Binding curve of histones H1dK52Q and H1tQ54K to Four-way junction DNA. (D) Binding curve of histones H1dK52Q and H1tQ54K to reconstituted mononucleosomes. The points in the above graphs represent the mean of three independent experiments and the error bars correspond to the standard deviation. (E) SDS gel profile showing the purified wild type and mutant histones. Sequences of the mutants are also indicated on the sides.

the results obtained with the reverse mutants of histones H1d and H1t provided strong evidence that the differences we have observed for the binding affinities of the histones are indeed due to the single amino acid change in the secondary DNA binding site within the globular domain. The secondary DNA binding site is conserved in all reported somatic histone variants in mammals (data not shown) whereas all reported histone H1ts harbor glutamine (Fig. 1B).

There has been considerable discussion in the literature on the importance of the second DNA binding site in the nucleosome binding property of the globular domain of histone H1. Hayes et al. [28] have also carried out a systematic mutational analysis of the second DNA binding site; but in contrast to Goytisolo et al. [16], they concluded that the second binding site is dispensable for binding of the globular domain to 5S DNA nucleosomal templates. However, they noted that mutation of Lys 40 or Arg 42 (in histones H10) altered the binding affinity of histone H10 towards DNA substrates to a small extent and mutation of Lys 40 also resulted in the loss of aggregation properties of histone H10. Histone H1t has a Gln instead of Lys at this position and we find a two-fold lesser binding affinity. Our results very clearly indicate that the individual residues of the secondary binding site, at least the Lys–Gln change observed in histone H1t, can influence histone H1

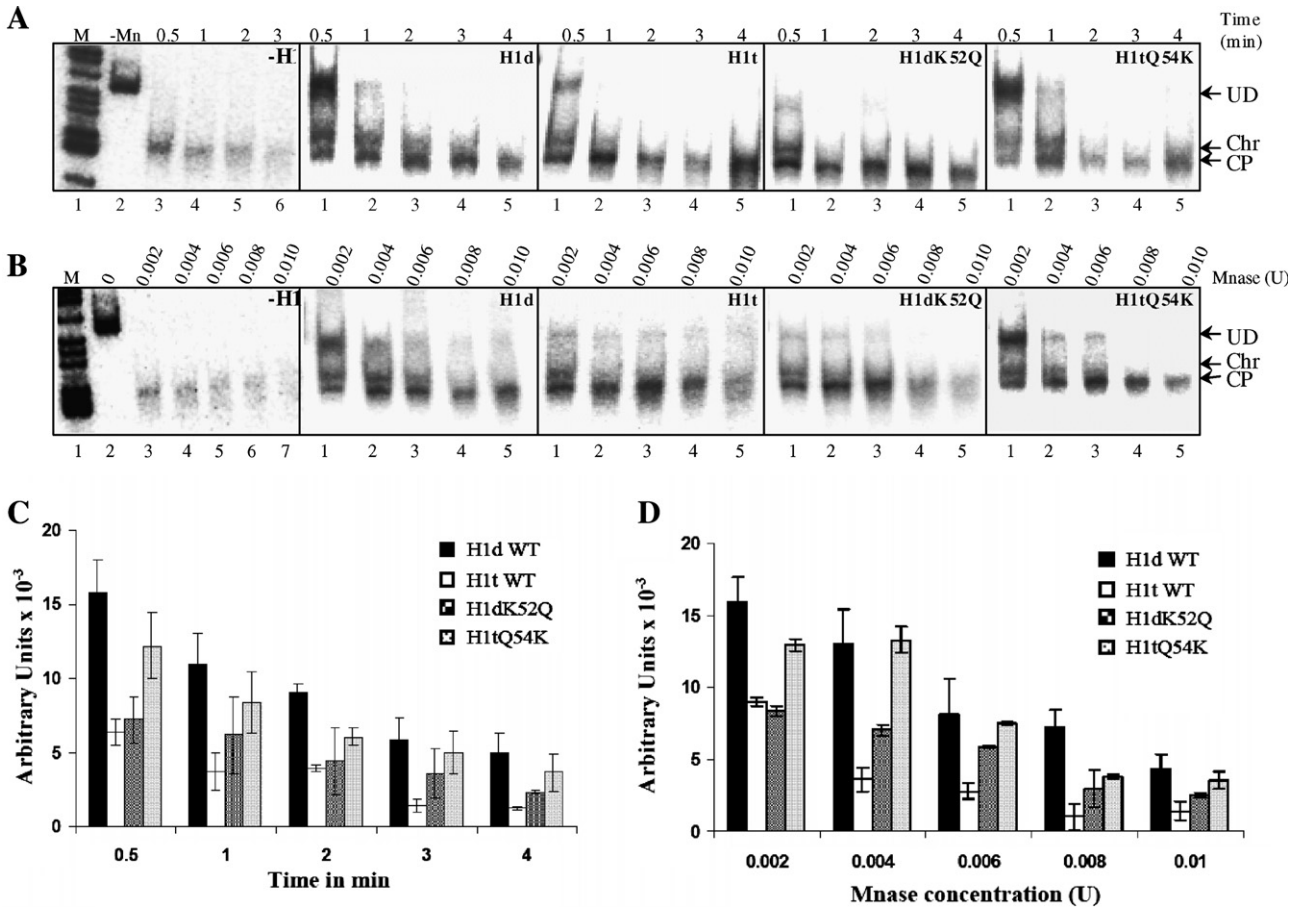

Fig. 5. Chromatosome stop assay with histone H1s. (A) Chromatosome stop on digestion of reconstituted nucleosome with 0.002 U of Mnase as a function of time. In no linker histone control (–H1), lane 1 corresponds to pBR Msp I marker; lane 2, no Mnase control; lanes 3–6 correspond to 0.5, 1, 2 and 3 min, respectively. In all other panels lanes 1–5 correspond to 0.5, 1, 2, 3 and 4 min, respectively. (B) Chromatosome stop as seen on digestion of reconstituted nucleosomes for a time period of 1 min as a function of Mnase concentration. In –H1 panel, lanes 3–7 correspond to 0.002 U, 0.004 U, 0.006 U, 0.008 U and 0.01 U of Mnase. In all other panels, lanes 1–5 correspond to the same amounts. In (A) and (B), M corresponds to pBR Msp I marker, UD, undigested mononucleosomes; Chr, chromatosome particle; CP, core particle. (C) and (D) are bar graphs representing the intensities of the 166 bp chromatosome band obtained from three independent experiments of (A) and (B), respectively.

binding, which in turn suggests that the secondary binding site is important and indispensable for H1 binding.

The biological significance of the appearance of histone H1t in a stage specific and tissue specific manner is still an enigma since there are three reports showing that spermatogenesis is not affected in histone H1t nullizygous mice [29–31]. Fantz et al. [31] have observed that H1t deficient mice have only 75% of total histone H1 when compared to normal mice. They have further suggested that the chromatin domains lacking histone H1t in the nullizygous mice might have an open chromatin conformation as in the histone H1t containing pachytene chromatin. It is also interesting to note that the globular domains of the newly discovered histone H1T2p and H1LSp have also glutamine residue in place of lysine in its secondary DNA binding site as in the case of histone H1t. The significance of this consistent replacement within the testis specific histone H1s also remains to investigated.

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References

[1] Allan, J., Cowling, G.J., Harbourne, N., Kattani, P., Craigie, R. and Gould, H. (1981) Regulation of the higher-order structure of chromatin by histones H1 and H5. J. Cell Biol. 90, 279–288.
[2] Lennox, R.W. and Cohen, L.H. (1984) The H1subtypes of mammals: metabolic characteristics and tissue distribution in: Histone Genes (Stein, G.S., Stein, J.L. and Marzulff, W.J., Eds.), pp. 373–395, John Wiley and Sons, NY.
[3] Tanaka, M., Hennebold, J.D., Macfarlane, J. and Adashi, E.Y. (2001) A mammalian oocyte-specific linker histone H1oo: homology with the genes for the oocyte-specific cleavage stage histone (Cs-H1) of sea urchin and the B4/H1M histone of the frog. Development 128, 655–664.
[4] Martianov, I., Braancorsini, S., Catena, R., Gansmuller, A.S., Kotaja, N., Parvinen, M., Sassone-Corsi, P. and Davidson, I. (2005) Polar nuclear localization of H1T2, a histone H1 varaint, required for spermatid elongation and DNA condensation during spermiogenesis. Proc. Natl. Acad. Sci. USA 102, 2808–2813.
[5] Tanaka, H., Iguchi, N., Isotani, A., Kitamura, K., Toyama, Y., Matsuoka, Y., Onishi, M., Masai, K., Mackawa, M., Toshimori, K., Okabe, M. and Nishimune, Y. (2005) HANP1/H1T2, a novel histone H1 like protein involved in nuclear formation and sperm fertility. Mol. Cell Biol. 25, 7107–7119.
[6] Yan, W., Ma, L., Burns, K.H. and Matsuk, M.M. (2003) H1LS is a spermatid specific linker histone like protein implicated in chromatin remodeling during mammalian spermiogenesis. Proc. Natl. Acad. Sci. USA 100, 10546–10551.

[7] Iguchi, N., Tanaka, H., Yamada, S., Nishimura, H. and Nishimune, Y. (2004) Control of mouse H1LS gene expression gene during spermatogenesis: Identification of regulatory element by transgenic mice. Biol. Reprod. 70, 1239–1245.
[8] DeLucia, F., Farone-Manella, M.R., D'Erne, M., Quesada, P., Caifa, P. and Farina, B. (1994) Histone induced condensation of rat testis chromatin: testis specific H1t versus somatic H1 variants. Biochem. Biophys. Res. Commun. 198, 32–39.
[9] Khadake, J.R. and Rao, M.R.S. (1995) DNA and chromatin condensing properties of rat testes H1a and H1t compared to those of rat liver H1bdec: H1t is a poor condenser of chromatin. Biochemistry 34, 15792–15801.
[10] Khadake, J.R. and Rao, M.R.S. (1997) Condensation of DNA and chromatin by an SPKK-containing octapeptide repeat motif present in the C-terminus of histone H1. Biochemistry 36, 1041–1051.
[11] Bharath, M.M., Chandra, N.R. and Rao, M.R.S. (2002) Prediction of an HMG-box fold in the C-terminal domain of histone H1: insights into its role in DNA condensation. Proteins 49, 71–81.
[12] Bharath, M.M., Ramesh, S., Chandra, N.R. and Rao, M.R.S. (2002) Identification of a 34 amino acid stretch in the C-terminal domain of histone H1as the DNA condensing domain by site directed mutagenesis. Biochemistry 41, 7617–7627.
[13] Lu, X. and Hansen, J.C. (2004) Identification of specific functional sub domains within the linker histone H10 C-terminal domain. J. Biol. Chem. 279, 8701–8707.
[14] Ramakrishnan, V., Finch, J.T., Graziano, V., Lee, P.L. and Sweet, R.M. (1993) Crystal structure of globular domain of histone H5 and its implications for nucleosomal binding. Nature 362, 219–223.
[15] Cerf, C., Lippens, G., Muyldermanns, S., Segers, A., Ramakrishnan, V., Wodak, S.J., Hallenga, K. and Wyns, L. (1993) Homo and Heteronuclear two dimensional NMR studies of the globular domain of histone H1: full assignment, tertiary structure and comparison with the globular domain of histone H5. Biochemistry 32, 11345–11351.
[16] Goytisolo, F.A., Gerchman, S.E., Yu, X., Rees, C., Graziano, V., Ramakrishnan, V. and Thomas, J.O. (1996) Identification of two DNA-binding sites on the globular domain of H5. EMBO J. 15, 3421–3429.
[17] Duggan, M.M. and Thomas, J.O. (2000) Two DNA-binding sites on the globular domain of histone H5 are required for binding to both bulk and 5S reconstituted nucleosomes. J. Mol. Biol. 304, 21–33.
[18] Bharath, M.M., Chandra, N.R. and Rao, M.R.S. (2003) Molecular modeling of the chromatosome particle. Nucl. Acids Res. 31, 4264–4274.
[19] Wisconsin Package Version 9.0. Genetics Computer Group (GCG), Madison, Wisc.
[20] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position specific gap penalties and weight matrix choice. Nucl. Acids Res. 22, 4673–4680.
[21] Rost, B. (1996) PHD: predicting one-dimensional protein structure by profile based neural networks. Meth. Enzymol. 266, 525–539.
[22] Bharath, M.M., Khadake, J.R. and Rao, M.R.S. (1999) Expression of rat liver histone H1d in *Escherichia coli* and its purification. Protein Express. Purif. 12, 38–44.
[23] Meetei, A.R. and Rao, M.R.S. (1999) Generation of multiple site-specific mutations in a single polymerase chain reaction product. Anal. Biochem. 264, 288–291.
[24] Nightingale, K.P., Pruss, D. and Wolffe, A.P. (1996) A single high affinity binding site for histone H1 in a nucleosome containing the *Xenopus borealis* 5S ribosomal RNA gene. J. Biol. Chem. 271, 7090–7094.
[25] Ura, K., Wolffe, A.P. and Hayes, J.J. (1994) Core histone acetylation does not block linker histone binding to a nucleosome including a *Xenopus borealis* 5S rRNA gene. J. Biol. Chem. 269, 27171–27174.
[26] Simpson, R.T. (1978) Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. Biochemistry 17, 5524–5531.
[27] Talasz, H., Sapojnikova, N., Helliger, W., Lindner, H. and Pusschendorf, B. (1998) In vitro binding of H1subtypes to nucleosomal organized mouse mammary tumor virus long terminal repeat promoter. J. Biol. Chem. 273, 32236–32243.
[28] Hayes, J.J., Kaplan, R., Ura, K., Prusss, D. and Wolffe, A.P. (1996) A putative DNA binding surface in the globular domain of a linker histone is not essential for specific binding to the nucleosome. J. Biol. Chem. 271, 25817–25822.
[29] Lin, Q., Sirotkin, A. and Skoultchi, A.I. (2000) Normal spermatogenesis in mice lacking the testis-specific linker histone H1t. Mol. Cell Biol. 113, 433–442.
[30] Drabent, B., Saffig, P., Bode, C. and Doenecke, D. (2000) Spermatogenesis proceed normally in mice without linker histone H1t. Histochem. Cell Biol. 20, 2122–2128.
[31] Fantz, D.A., Hatfield, W.R., Horvath, G., Kistler, M.K. and Kistler, W.S. (2001) Mice with a targeted disruption of the H1t gene are fertile and undergo normal changes in structural chromosomal proteins during spermatogenesis. Biol. Reprod. 64, 425–431.