Histone Gene Expression During Mammalian Spermatogenesis: Structural and Functional Aspects

Detlef DOENECKE & Birgit DRABENT

Institut für Biochemie und Molekulare Zellbiologie der Georg-August-Universität,
Humboldtallee 23, D-37073 Göttingen, Germany

ABSTRACT

The chromatin of spermatogenic cells undergoes structural rearrangements upon differentiation from spermatogonia to mature spermatozoa. During the haploid stages of mammalian spermatogenesis, histones are gradually replaced first by transition proteins and then by protamines. The histone fraction in chromatin of spermatogenic cells is composed of testis specific subtypes as well as such histone isoforms, which are also found in somatic tissues. The subtype patterns of all histone classes except H4 change in a stage specific manner during mammalian spermatogenesis. This implies that control mechanisms exist which regulate the cell type specific expression of the individual histone subtype genes. This control may be exerted at the transcriptional level as exemplified by functional studies at the H1t promoter. Regulation also may take place posttranslationally as demonstrated by the polyadenylation of part of the mRNA of spermatogenic cells.

RÉSUMÉ

Expression des gènes des histones pendant la spermatogenèse des Mammifères: aspects structuraux et fonctionnels

La chromatine des cellules spermatogénétiques subit des ré-arrangements structuraux pendant la différenciation progressant de la spermatogonie au spermatozoïde mûr. Pendant les stades haploïdes de la spermatogenèse des Mammifères, les histones sont remplacées graduellement d'abord par des protéines de transition puis par des protamines. La fraction des histones dans la chromatine des cellules spermatogénétiques est composée de sous-types spécifiques du testicule ainsi que d'isoformes des histones qui sont aussi rencontrées dans les tissus somatiques. Les sous-types de toutes les classes d'histones sauf H4 changent spécifiquement en fonction des étapes de la spermatogenèse des Mammifères. Ceci implique qu'il existe un mécanisme de contrôle, qui régule l'expression spécifique à chaque type cellulaire des gènes individuels de chaque sous-type d'histone. Ce contrôle peut être exercé au niveau transcriptionnel comme l'ont montré les études fonctionnelles sur le promoteur des H1t. La régulation peut aussi être post-traductionnelle ainsi que le montre la polyadénylation d'une partie des ARNm des cellules spermatogénétiques.

Histones are the basic chromosomal proteins of eukaryotic organisms. The histone protein familiy is composed of five protein species which have been classified on the basis of size and function. First, five different classes were defined by electrophoretic means and were termed H1,

DOENECKE, D. & DRABENT, B., 1995. — Histone gene expression during mammalian spermatogenesis: structural and functional aspects. *In*: JAMIESON, B. G. M., AUSIO, J., & JUSTINE, J.-L. (eds), Advances in Spermatozoal Phylogeny and Taxonomy. *Mém. Mus. natn. Hist. nat.*, **166** : 525-535. Paris ISBN : 2-85653-225-X.

H2A, H2B, H3 and H4 (for reviews, see [93, 95]). Two copies of each of the four histones H2A, H2B, H3 and H4 form the nucleosomal core. Therefore, they are summarily described as *core histones* in contrast to the H1 class proteins, which interact with the linker DNA connecting nucleosomal cores and are termed *linker histones*. Core and linker histones have been detected in nearly all eukaryotes [95]. The yeast *Saccharomyces cerevisiae* is an exception in having no linker histone [18], but its chromatin forms core nucleosomes and shows a subunit pattern with a regular spacing [90].

Histone protein patterns have been monitored during spermatogenesis in a broad spectrum of lower and higher eukaryotes [6, 7, 13, 16, 54, 62, 66, 67, 83, 85, 87]. Our group has concentrated on mammalian systems and has studied the structure and expression of somatic and testis-specific histone genes from man and mouse [2-4, 31, 32, 34-38]. In this contribution, structural and functional features of testicularly expressed histone genes and gene products will be discussed in relation to different stages of spermatogenesis (see Table 1).

TABLE 1. — Mammalian spermatogenic histone gene expression. Compilation of histone subtype proteins detectable at specific stages of sperm differentiation before replacement by transition proteins and finally by protamines (data from rat, mouse and man or from one or two only of these). Asterisks indicate expression data obtained using gene probes.

Histone subtype	Cellular stage of histone detection	Reference
H1a-e	any stage (predominantly H1a*,H1c) in part replaced by H1t (in pachytene spermatocytes) no H1 left in late spermatids	12, 13, 15, 33*, 62, 83, 85
H1°	spermatogonia* (then decreasing)	42*
H1t	pachytene spermatocytes* replacing main type H1 (a, c)	35*, 50*, 59, 60, 67
H2A	any stage (H2A.1>H2A.2), in part replaced by TH2A H2A subtype encoded by poly(A)$^+$-mRNA in spermatids*	12, 67 71*
H2A.X	spermatogonia*	64*, 67
H2A.Z	spermatogonia (low), slight increase in pachytene spermatocytes	53, 67
TH2A	pachytene spermatocytes* partly replacing H2A	56*, 63, 67, 69
H2B	any stage, major part replaced by TH2B specific subtype in spermatids* (extended C-terminus)	12, 67 70*
TH2B	pachytene spermatocytes* (and later stages)	12, 57, 58*
H3	any stage (H3.2>H3.1)	12, 67
TH3	spermatogonia, absent during later stages	89
H3.3	any stage until spermatids, predominantly in spermatogonia	9, 67, 91
H4	any stage H1t associated H4* gene transcribed in pachytene spermatocytes	12, 67, 68 94*

OBSERVATIONS AND DISCUSSION

Testicular histone subtypes

The H1 linker histone family of mammals comprises several variant isoforms. The most detailed analysis of the H1 complement has been done in human and murine chromatin in several cell and tissue types. In humans, five main type H1 histone genes have been described [3] in addition to the gene encoding H1° [31], which is a histone confined to highly differentiated cell types, and the highly conserved H1t gene [34], which is only expressed in testicular cells. H1t protein sequences are known from man [34], other primates [59], mouse [35], rat [22] and boar [21]. The H1t protein is confined to male germ cells and is not a general meiosis specific variant [65]. As in humans, five different mouse H1 proteins (or genes) [3, 33, 62, 88, 89, 97] plus H1° [4] and H1t [35] were described. As yet, only one rat main type H1 gene [23, 36], the H1t gene [22] and a partial H1° cDNA sequence [17] have been described. The rat H1t gene and gene promoter structures have been intensely studied [49-51] and will be discussed below. The H1t fraction of the overall testicular H1 complement amounts to about 25% [78], and somatic type isoforms [62] and the H1° fraction [84, 87] constitute the remaining part of the H1 histone moiety in testicular chromatin. In pachytene spermatocytes and later stages H1t may comprise a much higher fraction [13, 67].

Core histone isoforms, which are restricted to testicular cells, have been described in several species. A pair of testicularly expressed genes consisting of an H2A and an H2B gene was described by HUH *et al.* [55]. These genes appear to code for the previously described testicular subtypes TH2A and TH2B, respectively [10, 58, 92, 98]. In addition to main type H2A and H2B isoforms, two H2A subtypes, which are replication independent, i.e. H2A.Z and H2A.X [53, 64], have been described. H2A.X is enriched in testicular chromatin, whereas H2A.Z is uniformly found in most somatic tissues [53]. A testicular subtype of H3 (TH3) has been isolated from rat testis [91]. Its unique amino acid composition (including three cysteine residues) indicates structural differences compared with all other known H3 subtypes, but as yet no TH3 gene has been identified in any mammalian genome. The replication independent H3 subtype H3.3 is also expressed during spermatogenesis. For example, H3.3 has been observed in spermatid stages of spermiogenesis [69], but it is also present at earlier stages of spermatogenesis [67, 91]. H4 is the most conserved of all histone classes. Its 102 amino acid sequence is strictly maintained in all mammalian species. This even applies to H4 genes which are differentially expressed. For example, the human, rat or mouse H1t genes are located near testicularly expressed H4 genes which code for the same H4 amino acid sequences as other H4 genes from the same species.

Organization of mammalian histone gene clusters

The majority of histone genes in the murine and human genomes is clustered at specific chromosomal sites. Except the H1° gene, all known human H1 genes and surrounding core histone genes are located on chromosome 6 [3]. A minor portion of core histone genes maps to chromosome 1 [46], and the solitary H1° gene is located on the long arm of chromosome 22 [3]. The situation in the murine genome appears to be similar, since a major histone gene cluster including the H1t gene has been mapped to chromosome 13 [26, 75], and the murine H1° gene is on chromosome 15 in a region which is syntenic with the region on the human chromosome 22, where the human H1° gene is located [3, 11].

The human H1t gene, which is expressed in pachytene spermatocytes (see below) forms part of the major gene cluster on chromosome 6, which also contains the other H1 genes [3]. Thus, the generation of H1 histone patterns, which are characteristic for cells of specific stages of spermatogenesis, must depend on a differential regulation of the genes within that major cluster. In addition, the expression of the H1° gene, which appears to be developmentally regulated

during differentiation of several cell types [99], must undergo tissue-specific control. It is preferentially expressed during early stages of spermatogenesis [42] and in somatic cells it mainly appears upon terminal differentiation [99].

The genes coding for the testicularly expressed histones TH2A, TH2B and TH3 have not yet been mapped to specific chromosomal sites. They also may form part of the major histone gene cluster. GRIMES *et al.* [49] have shown that an H4 gene, which is located near the rat H1t gene, is testicularly expressed. It has the same primary structure as other mammalian H4 proteins. On the basis of its variant nucleotide sequence and testicular expression, this gene may be termed H4t [49, 94]. Its association with the H1t gene, which is located within the major cluster of somatically expressed histone genes, implies that it is not a solitary gene. In contrast to the H1t gene, expression of this neighbouring H4 (H4t) gene is not confined to spermatogenic cells, but its mRNA also has been detected in a rat myeloma cell line.

Cell type specific histone patterns at different stages of spermatogenesis

The H1 patterns of different somatic cell types or germ cells are not uniform, but vary in their H1 subtype composition. In several mammalian species, five main type H1 protein species (termed H1a-H1e) were described [62]. In rat testes, the subtypes H1a-H1e can be detected (H1a and H1c predominating) during all stages of spermatogenesis until the primary spermatocyte stage [12, 62]. Similarly, the subtypes H1a and H1c predominate in mouse germ cells until the meiotic prophase [62]. Immunocytochemical analysis of murine tubuli seminiferi showed the greatest level of reactivity in primary spermatocyte nuclei using antibodies against H1a [79]. Developmental studies showed that the first expression of the H1a gene occurs in 7 day old mice at a stage when intermediate and B type spermatogonia appear [79]. *In situ* hybridizations with human testis detected the mRNA coding for human H1.1 (equals H1a according to [76]) until the stage of round spermatids [14, 15]. Thus, the subtype H1a appears to be a major constituent in the chromatin of mammalian germ cells [13, 67]. In addition, the subtypes H1b, c and d contribute to the germ cell chromatin [62, 67, 78].

The H1 subtype H1° has been described in unfractionated mammalian testis cell preparations [83, 85, 86]. The predominant expression of the H1° gene in spermatogonia was suggested by promoter studies of GARCIA-IGLESIAS *et al.* [42]. In that work, the H1° promoter was ligated upstream of a ß-galactosidase gene and the expression of this construct was monitored in transgenic mice. The analysis showed that the promoter was used in several tissues, such as specific cell types in kidney, brain and testis. Testicular mRNA synthesis was mostly confined to spermatogonia, but immunofluorescence studies with H1° antibodies indicated expression in Sertoli cells, too. Thus, expression of the H1° gene may be confined to early stages of spermatogenesis, but somatic cells in the testis also express the H1° gene.

The H1t protein is absent from spermatogonia and is first detected in pachytene spermatocytes [35, 60, 69]. This has been demonstrated at the protein level in chromatin from mouse and rat cells fractionated by elutriation centrifugation [44]. After the cloning of the genes coding for the human, murine and rat H1t proteins [22, 34, 35], Northern blot and *in situ* hybridization analysis has confirmed these protein data showing that the mRNA is only found in pachytene spermatocytes [60] whereas the proteins are preserved in the subsequent stages until histone replacement by transition proteins [69].

The major change in chromatin structure during the meiotic prophase is also evident in the H2A/H2B class of histones. The subtypes TH2A and TH2B both become first detectable in pachytene spermatocytes of rat and mouse [20, 67, 78, 92]. The subtype H2A.X, which, like H1°, is a non replication-dependent histone [72, 96], has been detected in type A spermatogonia [64]. Expression of a modified H2B protein has been found during mouse spermiogenesis [70]. In a cDNA library constructed from spermatid RNA, an H2B cDNA sequence was observed which was extremely similar to other mouse H2B gene sequences, but the C-terminus coded for

12 additional amino acids, 7 of which were hydrophobic. Northern blots with RNA from other tissues indicated that this transcript was testis-specific [70]. Similarly, a polyadenylated H2A mRNA was detected in mouse round spermatids [71].

The histone to protamine transition during human spermiogenesis does not result in a complete removal of all histones [43, 52]. About 15% of the human sperm DNA appears to remain associated with core histones, i.e. mainly with H2A (H2A.X and trace amounts of H2A.Z), several H2B isotypes, H3.1, H3.3 and highly acetylated H4 [43]. In contrast to these remaining core histones, no association of any H1 subtype with mature sperm chromatin has been observed.

The gradual changes of the core and linker histone moieties during the development of male germ cells and the changes in chromatin morphology and gene activity suggest a functional role for the individual histone subtypes. However, correlations between specific structural features of histones and functional differences have not yet been established in any somatic or germ cell system. H1°, which appears to be confined to early stages of spermatogenesis, is correlated with terminal differentiation in several cell types (for review, see [99]). Its avian countertpart H5 is confined to the condensed, transcriptionally inactive nuclei of avian red blood cells [5]. The high arginine content [30, 31] of the H5 histone is considered as one of the reasons for its condensing capacity. Compared with the other H1 subtypes, H1t is also enriched in arginine, but DE LUCIA *et al.* [29] have shown by circular dichroism analysis that H1t has a lower condensing capacity than the other H1 subtypes. Thus, H1t may even contribute to activating effects in the chromatin of developing germ cells rather than repressing nuclear activity. It may thus help to decondense the chromatin structure for the specific needs of the meiotic and haploid stages of germ cell development.

Postsynthetic histone modifications

Posttranslational modifications of histone proteins have been primarily observed at spermatid stages of spermatogenic cell development. Recently, the phosphorylation of H1t in elongating spermatids has been described [69]. In the same study, which used vitamin A as a means to synchronize rat seminiferous epithelia into few stages of spermiogenesis, additional bands of H2A.1, H2A.2 and TH2A were observed and were interpreted as postsynthetic modifications. A complex pattern of phosphorylation of the H2A.X subtype has been observed in murine testicular cells [45]. Another type of histone modification is the conjugation with ubiquitin. This has been described for H2A histones during rooster spermatogenesis [1].

The most impressive modification of histones during spermatogenesis is their hyperacetylation. This modification of the H4 histone structure is correlated with a broad spectrum of cellular processes, including transcriptional control and chromatin assembly (for review, see [95]). H4 hyperacetylation occurs in elongating spermatids [47, 48, 68]. This is the stage when displacement of histones by transition proteins begins [69, 73]. Thus, the association of highly acetylated H4 with the stage of histone displacement in rat spermatids is in agreement with the idea that reducing the positive charge of specific lysine residues may help to displace histones from chromatin during spermiogenesis.

Regulation of testicular histone gene expression

The location of spermatogenesis related histone genes within clusters of somatic histone subtype genes implies that control steps discriminate between the different member genes of the gene cluster. This control may take place at the transcriptional level, but also posttranscriptionally, i.e. during processing of the primary transcript or by influencing the stability of specific histone mRNAs (for review, see [74]). At the transcriptional level, promoter structures of specific histone genes may contain sequence motifs where interaction with germ cell-specific transcription factors controls the specific expression of the respective genes.

The mechanism of H1 histone gene regulation in *somatic* cells is not yet fully understood. Sequence analysis of H1 gene promoters in all vertebrate systems studied revealed that the heptanucleotide AAACACA is conserved at a position 100 nucleotides upstream of the transcription start site [25, 27, 28]. Functional studies indicated the involvement of this H1-box in the S-phase-dependent expression of H1 genes [27, 28, 61], but variants of this sequence motif have been observed [38]. A second sequence element, which is involved in the regulation of H1 genes, is the CCAAT box, which is the binding site for an H1-specific regulatory factor [41]. The sequence analysis of the rat, human and murine H1t promoters revealed that their sequences contained all main features of S-phase-dependent H1 genes: TATA-box, GC-rich element, CCAAT-motif and H1-box [22, 34, 35, 50, 51]. Thus, the known regulatory elements within H1t promoter structures apparently do not reflect the fact that the H1t gene is not transcribed during DNA replication, but at the pachytene stage of the meiotic prophase. GRIMES and coworkers [50, 51] searched for a testis-specific element and defined the palindromic hexanucleotide CCTAGG, which is located between the GC-rich element and the CCAAT-box of the rat H1t promoter as the testis-specific promoter element [50, 51]. This element was identified as the site of interaction of testis-specific DNA-binding proteins at the promoter in pachytene spermatocytes [51]. Further support for a functional role of this sequence element may be derived from the human H1t promoter, where this sequence motif is conserved at the same site [32, 34]. The palindromic arrangement, however, may not be mandatory, since the mouse H1t promoter shows a varied element, CCTGGG, at the same location [35].

The rat TH2A and TH2B genes are grouped together, and they are divergently transcribed from a joint promoter region of about 240 nucleotides in between the two genes [56-58]. In both directions, TATA- and CCAAT-boxes are located upstream of the two genes. In addition, the TH2B gene promoter contains the OctI element ATTTGCAT, which is a characteristic regulatory element in all H2B gene promoters [40] but also in control regions of several other genes. For example, variant Oct factors binding to such elements have been detected during mouse embryogenesis and are specifically expressed in germline cells [81]. In conclusion, the promoter arrangement of the TH2B gene does not vary from consensus H2B promoter structures and it does not reflect the replication-independent, testis-specific expression of this histone gene. Functional studies with the TH2B promoter in fibroblast cells revealed that the CCAAT- and octamer elements of this promoter are involved in the S-phase dependent expression of the TH2B gene when transfected into these *somatic* cells [56-58]. Subsequent studies showed that differential methylation at specific sites of the TH2B promoter contributes to the tissue-specific transcription of this TH2B gene [20] and that a repressor protein specific for the rat TH2B gene was present during early stages of spermatogenesis [63].

The gene coding for TH3, which has been described as a testis-specific H3 subtype in rat spermatogonia [91], has not yet been detected. Thus, no data on cell specific regulation of testicular H3 histones exist. As mentioned above, GRIMES *et al.* [49] have shown that an H4 gene is closely associated with the rat H1t gene. S1 nuclease analysis has shown that this particular H4 gene is transcribed in the testis predominantly during the pachytene stage, but it is also expressed in a tumor cell line. This is in contrast to the neighbouring H1t gene, which is solely transcribed in pachytene spermatocytes [50, 94].

The control of histone gene expression is not restricted to transcriptional regulation (for review, see [74]). Processing of the primary transcript and mRNA stability of replication dependent histone gene products depend on the presence of a dyad symmetry element at the 3' end of the mRNA, which is not polyadenylated [8, 82]. The only exceptions from this rule are the S phase-independent replacement histone variants, such as H1°, H3.3, H2A.Z or H2A.X, which are all encoded by polyadenylated histone mRNAs. Remarkably, the H1t genes of rat, mouse and man show the same dyad symmetry elements as replication dependent histone genes and the mRNAs are non polyadenylated.

Posttranscriptional regulation of testicular histone gene expression

As mentioned above, main type, S phase-dependent histone mRNAs in somatic cells are poly(A)$^-$ in contrast to the mRNAs encoding the replacement histone subtype mRNAs. In addition to these specific subtype mRNAs, polyadenylated testicular histone mRNAs have been described [39]. These are at least in part derived from genes which are transcribed to poly(A)$^-$-mRNA in somatic cells. A poly(A)$^+$ histone H2B mRNA with an extended reading frame and a consensus AAUAAA polyadenylation signal has been detected in mouse spermatids [70]. Recently, a polyadenylated H2A gene transcript was found in murine round spermatids. In this case, the poly(A) tail was not preceded by the somatic AAUAAA signal sequence [71]. In a detailed analysis of histone mRNAs in chicken spermatids, CHALLONER *et al.* [19] detected an H2B mRNA subpopulation, which was polyadenylated despite the fact that the histone mRNA was derived from a gene which is transcribed as a poly(A)$^-$-mRNA in somatic cells. The transcript from this same gene was elongated by 26 or 28 nucleotides beyond the histone mRNA consensus termination site, and a poly(A) tail was added to this elongated mRNA.

A major step in histone gene regulation is the control of mRNA degradation [74]. This has not been specifically studied in testicular histone gene expression, but the addition of poly(A) tails to part of the histone mRNA population during spermatogenesis suggests that it is a means to increase the stability of this mRNA, which is either synthesized at post-meiotic stages or is synthesized at early spermatogenesis and is preserved for later stages of development, when a certain pool of mRNAs for histone replacement may be needed.

Conclusions

Modulation of the chromatin structure during spermatogenesis requires changed patterns of histone proteins and histone modifications which contribute to restructuring of chromatin and to the transition towards the inactivation of the genome in generating the condensed genome of mature sperm. Specific histone subtypes, which differ from their somatic counterparts have been described for all histone classes except H4. The most drastic changes in histone gene expression and chromatin restructuring occur during the meiotic prophase, when specific subtypes of H1, H2A and H2B, i.e. H1t, TH2A and TH2B, are synthesized. These testis specific isoforms remain associated with the chromatin of cells during the haploid stages of sperm cell differentiation. At this spermiogenesis period, remodelling of chromatin before the final deposition of protamines may require a specific chromatin structure which is accessible for regulatory factors such as non-histone proteins and for transition proteins replacing the histone moiety. This specific chromatin structure may be established by specific subsets of core and linker histones and by their posttranslational modification.

ACKNOWLEDGEMENTS

Work performed in the laboratory of the authors was supported by a grant from the Deutsche Forschungsgemeinschaft.

REFERENCES

1. AGELL, N., CHIVA, M. & MEZQUITA, C., 1983. — Changes in nuclear content of protein conjugate histone H2A-ubiquitin during rooster spermatogenesis. *FEBS Letters*, **155**: 209-212.

2. ALBIG, W., KARDALINOU, E., DRABENT, B., ZIMMER, A. & DOENECKE, D., 1991. — Isolation and characterization of two human H1 histone genes within clusters of core histone genes. *Genomics*, **10**: 940-948.

3. ALBIG, W., DRABENT, B., KUNZ, J., KALFF-SUSKE, M., GRZESCHIK, K.H. & DOENECKE, D., 1993. — All known human histone H1 genes except the H1° gene are clustered on chromosome 6. *Genomics*, **16**: 649-654.

4. ALONSO, A., BREUER, B., BOUTERFA, H. & DOENECKE, D., 1988. — Early increase in histone H1° mRNA during differentiation of F9 cells to parietal endoderm. *The EMBO Journal*, **7**: 3003-3008.

5. APPELS, R. & WELLS, J. R. E., 1972. — Synthesis and turnover of DNA-bound histone during maturation of avian red blood cells. *Journal of Molecular Biology*, **70**: 425-434.

6. AUSIO, J. & K.E. VAN HOLDE, 1987. — A dual chromatin organization in the sperm of the bivalve mollusc *Spisula solidissima*. *European Journal of Biochemistry*, **165**: 363-371.
7. AUSIO, J., 1988. — An unusual cysteine-containing histone H1-like protein and two protamine-like proteins are the major nuclear proteins of the sperm of the bivalve mollusc *Macoma nasuta*. *Journal of Biological Chemistry*, **263**: 10141-10150.
8. BIRNSTIEL, M. L., BUSSLINGER, M. & STRUB, K., 1985. — Transcription termination and 3' processing: the end is in site! *Cell*, **41**: 349-359.
9. BHATNAGAR, Y. M. & BELLVE, A. R., 1978. — Two-dimensional electrophoretic analysis of major histone species and their variants from somatic and germ-line tissue. *Analytical Biochemistry*, **86**: 754-760.
10. BHATNAGAR, Y. M., FAULKNER, R. D. & MCCULLAR, M. K., 1985. — Biochemical and immunological characterization of an H2A variant of the mouse testis. *Biochimica et Biophysica Acta*, **827**: 14-22.
11. BRANNAN, C. I., GILBERT, D. J., CECI, J. D., MATSUDA, Y., CHAPMAN, V. M., MERCER, J. A., EISEN, H., JOHNSTON, L.A., COPELAND, N. G., JENKINS, N. A., 1992. — An interspecific linkage map of mouse chromosome 15 positioned with respect to the centromere. *Genomics*, **13**: 1075-1081.
12. BROCK, W. A., TROSTLE, P. K. & MEISTRICH, M. L., 1980. — Meiotic synthesis of testis histones in the rat. *Proceedings of the National Academy of Sciences USA*, **77**: 371-375.
13. BUCCI, L. R., BROCK, W. A. & MEISTRICH, M. L., 1982. — Distribution and synthesis of histone H1 subfractions during spermatogenesis in the rat. *Experimental Cell Research*, **140**: 111-118.
14. BURFEIND, P., HOYER-FENDER, S., DOENECKE, D., TSAOUSIDOU, S. & ENGEL, W., 1992. — Expression of a histone H1 gene (H1.1) in human testis and Hassall's corpuscles of the thymus. *Thymus*, **19**: 245-251.
15. BURFEIND, P., HOYER-FENDER, S., DOENECKE, D., HOCHHUTH, C. & ENGEL, W., 1994. — Expression and chromosomal mapping of the gene encoding the human histone H1.1. *Human Genetics*, **94**: 633-639.
16. CARLOS, S., JUTGLAR, L., BORRELL, I., HUNT, D. F. & AUSIO, J., 1993. — Sequence and characterization of a sperm specific histone H1-like protein of Mytilus californianus. *Journal of Biological Chemistry*, **268**: 185-194.
17. CASTIGLIA, D., GRISTINA, R., SCATURRO, M. & DI LIEGRO, I., 1993. — Cloning and analysis of cDNA for rat histone H1°. *Nucleic Acids Research*, **21**: 1674.
18. CERTA, U., COLAVITO-SHEPANSKI, M. & GRUNSTEIN, M., 1984. — Yeast may not contain histone H1: the only known "histone H1-like protein" in *Saccharomyces cerevisiae* is a mitochondrial protein. *Nucleic Acids Research*, **12**: 7975-7985.
19. CHALLONER, P. B., MOSS, S. B. & GROUDINE, M., 1989. — Expression of replication dependent histone genes in avian spermatids involves an alternate pathway of mRNA 3'-end formation. *Molecular and Cellular Biology*, **9**: 902-913.
20. CHOI, Y. C. & CHAE, C. B., 1991. — DNA hypermethylation and germ cell specific expression of testis-specific H2B histone gene. *Journal of Biological Chemistry*, **266**: 20504-20511.
21. COLE, K. D., YORK, R. G. & KISTLER, W. S., 1984. — The amino acid sequence of boar H1t, a testis-specific H1 histone variant. *Journal of Biological Chemistry*, **259**: 13695-13702.
22. COLE, K. D., KANDALA, J. C. & KISTLER, W. S., 1986. — Isolation of the gene for the testis-specific H1 histone variant H1t. *Journal of Biological Chemistry*, **261**: 7178-7183
23. COLE, K. D., KANDALA, J. C., KREMER, E. & KISTLER, W. S., 1990. — Isolation of a genomic clone encoding the rat histone variant H1d. *Gene*, **89**: 265-269.
24. COLE, R. D., 1987. — Microheterogeneity in H1 histones and its consequences. *International Journal of Peptide and Protein Research*, **30**: 433-449.
25. COLES, L. S. & WELLS, J. R. E., 1985. — An H1-histone gene-specific 5' element and evolution of H1 and H5 genes. *Nucleic Acids Research*, **13**: 585-594.
26. COX, D. R., GROPPI, V. E., BIBER, D., SITTMAN, D. B., COFFINO, P. AND MARZLUFF, W. F., 1984. — Assignment of murine histone genes to mouse chromosome 13. *Cytogenetics and Cell Genetics*, **37**: 443.
27. DALTON, S. & WELLS, J. R. E., 1988. — A gene specific promoter element is required for optimal expression of the histone H1 gene in S-phase. *EMBO Journal*, **7**: 49-56.
28. DALTON, S. & WELLS, J. R. E., 1988. — Maximal binding levels of an H1 histone gene-specific factor in S-phase correlate with maximal H1 gene transcription. *Molecular and Cellular Biology*, **8**: 4576-4578.
29. DE LUCIA, F., FARAONE-MENNELLA, M. R., D'ERME, M., QUESADA, P., CAIAFA, P. & FARINA, B., 1994. — Histone induced condensation of rat testis chromatin: testis-specific H1t versus somatic H1 variants. *Biochemical and Biophysical Research Communications*, **198**: 32-39.
30. DOENECKE, D. & TONIES, R., 1984. — Conserved dyad symmetry structures at the 3' ends of H5 histone genes. Analysis of the duck H5 gene. *Journal of Molecular Biology*, **178**: 121-135.

31. DOENECKE, D. & TONJES, R., 1986. — Differential distribution of lysine and arginine residues in the closely related histones H1° and H5. Analysis of a human H1° gene. *Journal of Molecular Biology*, **187**: 461-464.

32. DOENECKE, D., ALBIG, W., BOUTERFA, H. & DRABENT, D., 1994. — Organization and expression of H1 histone and H1 replacement histone genes. *Journal of Cellular Biochemistry*, **54**: 423-431.

33. DONG, Y., SIROTKIN, A. M., YANG, Y. S., BROWN, D. T., SITTMAN, D. B. & SKOULTCHI, A. I., 1994. — Isolation and characterization of two replication-dependent mouse H1 histone gens. *Nucleic Acids Research*, **22**: 1421-1428.

34. DRABENT, B., KARDALINOU, E. & DOENECKE, D., 1991. — Structure and expression of the human gene encoding testicular H1 histone (H1t). *Gene*, **103**: 263-268.

35. DRABENT, B., BODE, C. & DOENECKE, D., 1993. — Structure and expression of the mouse testicular H1 histone gene (H1t). *Biochimica et Biophysica Acta*, **1216**: 311-313.

36. DRABENT, B., KUNZ, C. & DOENECKE, D., 1993. — A rat histone H2B pseudogene is closely associated with the histone H1d gene. *Biochimica et Biophysica Acta*, **1172**: 193-196.

37. EICK, S., NICOLAI, M., MUMBERG, D., 1989. — Human H1 histones: conserved and varied sequence elements in two H1 subtype genes. *European Journal of Cell Biology*, **49**: 110-115.

38. EILERS, A., BOUTERFA, H., TRIEBE, S. & DOENECKE, D., 1994. — Role of a distal promoter element in the S-phase control of the human H1.2 histone gene transcription. *European Journal of Biochemistry*, **223**: 567-574.

39. FAULKNER, R. D., WHISENANT, E. C. & BHATNAGAR, Y. N., 1986. — Histone mRNAs of the mouse testis. *Biochemical and Biophysical Research Communications*, **136**: 1116-1123.

40. FLETCHER, C., HEINTZ, N. & ROEDER, R. G., 1987. — Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2B gene. *Cell*, **51**: 773-781.

41. GALLINARI, P., LABELLA, F. & HEINTZ, N., 1989. — Characterization and purification of H1TF2, a novel CCAAT-binding protein that interacts with a histone H1-subtype specific consensus element. *Molecular and Cellular Biology* **9**: 1566-1575.

42. GARCIA-IGLESIAS, M. J., RAMIREZ, A., MONZO, M., STEUER, B., MARTINEZ, J. M., JORCANO, J. L. & ALONSO, A., 1993. — Specific expression in adult mice and post-implantation embryos of a transgene carrying the histone H1° regulatory region. *Differentiation* **55**: 27-35.

43. GATEWOOD, J. M., COOK, G. R., BALHORN, R., SCHMID, C. W. & BRADBURY, E. M., 1990. — Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. *Journal of Biological Chemistry*, **265**: 20662-20666.

44. GRABSKE, R., LAKE, S., GLEDHILL, B. L. & MEISTRICH, M. L., 1975. — Centrifugal elutriation: separation of spermatogenic cells on the basis of sedimentation velocity. *Journal of Cellular Physiology*, **86**: 177-190.

45. GREEN, G. R., PATEL, J. C., HECHT, N. B. & POCCIA, D. L., 1991. — A complex pattern of H2A phosphorylation in the mouse testis. *Experimental Cell Research*, **195**: 8-12.

46. GREEN, L. G., VAN ANTWERPEN, R., STEIN, J., STEIN, G., TRIPUTTI, P., EMANUEL, B., SELDEN, J. & CROCE, C., 1984. — A major histone gene cluster on the long arm of chromosome 1. *Science*, **226**: 838-840.

47. GRIMES, S. R., CHAE, C. B. & IRVIN, J. L., 1975. — Acetylation of histones of rat testis. *Archives of Biochemistry and Biophysics*, **168**: 425-435.

48. GRIMES, S. R. & HENDERSON, N., 1984. — Hyperacetylation of histone H4 in rat testis spermatids. *Experimental Cell Research*, **152**: 91-97.

49. GRIMES, S., WEISZ-CARRINGTON, P., DAUM, H., SMITH, J., GREEN, L., WRIGHT, K., STEIN, G. & STEIN, J., 1987. — A rat histone H4 gene closely associated with the testis-specific H1t gene. *Experimental Cell Research*, **173**: 534-545.

50. GRIMES, S. R., WOLFE, S. A., ANDERSON, J. V., STEIN, G. S. & STEIN, J. L., 1990. — Structural and functional analysis of the rat testis-specific H1t gene. *Journal of Cellular Biochemistry*, **44**: 1-17.

51. GRIMES, S. R., WOLFE, S. A. & KOPPEL, D. A., 1992. — Temporal correlation between the appearance of testis-specific DNA-binding proteins and the onset of transcription of the testis-specific histone H1t gene. *Experimental. Cell Research*, **201**: 216-224.

52. GUSSE, M., SAUTIÈRE, P., BELAICHE, D., MARTINAGE, A., ROUX, C., DADOUNE, J. P. & CHEVAILLIER, P., 1986. — Purification and characterization of nuclear basic proteins of human sperm. *Biochimica et Biophysica Acta*, **884**: 249-257.

53. HATCH, C. L. & BONNER, W. M., 1988. — The human histone H2A.Z gene. *Journal of Biological Chemistry*, **265**: 15211-15218.

54. HENTSCHEL, C. C. & BIRNSTIEL, M. L., 1981. — The organization and expression of histone gene families. *Cell*, **25**: 301-313.

55. HUH, N. E., HWANG, I., LIM, K., YOU, K.H. & CHAE, C. B., 1991. — Presence of a bi-directional S phase-specific transcription regulatory element in the promoter shared by testis-specific TH2A and TH2B histone genes. *Nucleic Acids Research*, **19**: 93-98.

56. HWANG, I. & CHAE, C. B., 1989. — S-phase specific transcription regulatory elements are present in a replication-independent testis-specific H2B histone gene. *Molecular and Cellular Biology*, **9**: 1005-1013.

57. HWANG, I., LIM, K. & CHAE, C. B., 1990. — Characterization of the S-phase-specific transcription regulatory elements in a DNA replication-independent testis-specific H2B (TH2B) histone gene. *Molecular and Cellular Biology*, **10**: 585-592.

58. KIM, Y. J., HWANG, I, TRES, L. L.., KIERSZENBAUM, A. L. & CHAE, C. B., 1987. — Molecular cloning and differential expression of somatic and testis-specific H2B histone genes during rat spermatogenesis. *Developmental Biology*, **124**: 23-34.

59. KOPPEL, D. A., WOLFE, S. A., FOGELFELD, L. A., MERCHANT, P. S., PROUTY, L. & GRIMES, S. R., 1994. — Primate testicular histone H1t genes are highly conserved and the human H1t gene is located on chromosome 6. *Journal of Cellular Biochemistry*, **54**: 219-230.

60. KREMER, E. J. & KISTLER, W. S., 1991. — Localization of mRNA for testis-specific histone H1t by in situ hybridization. *Experimental Cell Research*, **197**: 330-332.

61. LABELLA, F., GALLINARI, P., MCKINNEY, J. & HEINTZ, N., 1989. — Histone H1 subtype-specific consensus elements mediate cell cycle-regulated transcription in vitro. *Genes & Development*, **3**: 1982-1990.

62. LENNOX, R. W. & COHEN, H., 1984. — The alterations in H1 histone complement during mouse spermatogenesis and their significance for H1 subtype function. *Developmental Biology*, **103**: 80-84.

63. LIM, K. & CHAE, C. B., 1992. — Presence of a repressor protein for testis-specific H2B (TH2B) histone gene in early stages of spermatogenesis. *Journal of Biological Chemistry*, **267**: 15271-15273.

64. MANNIRONI, C., BONNER, W. M. & HATCH, C. L., 1989. — H2A.X, a histone isoprotein with a conserved C-terminal sequence is encoded by a novel mRNA with both DNA replication type and poly(A) 3' processing signals. *Nucleic Acids Research*, **17**: 9113-9136.

65. MARKOSE, E. R. & RAO, M. R. S., 1989. — Testis-specific histone H1t is truly a testis-specific variant and not a meiotic-specific variant. *Experimental Cell Research*, **182**: 279-283.

66. MAXSON, R., COHN, R., KEDES, L. & MOHUN, T., 1983. — Expression and organization of histone genes. *Annual Review of Genetics*, **17**: 239-277.

67. MEISTRICH, M. L., BUCCI, L. R., TROSTLE-WEIGE, P. K. & BROCK, W. A., 1985. — Histone variants in rat spermatogonia and primary spermatocytes. *Developmental Biology*, **112**: 230-240.

68. MEISTRICH, M. L., TROSTLE-WEIGE, P. K., LIN, R., BHATNAGAR, Y. M. & ALLIS, C. D., 1992. — Highly acetylated H4 is associated with histone displacement in rat spermatids. *Biology of Reproduction*, **51**: 334-344.

69. MEISTRICH, M. L., TROSTLE-WEIGE, P. K. & VAN BEEK, M. E. A. B., 1994. — Separation of specific stages of spermatids from Vitamin-A-synchronized rat testes for assessment of nucleoprotein changes during spermatogenesis. *Biology of Reproduction*, **51**: 334-344.

70. MOSS, S. B., CHALLONER, P. B. & GROUDINE, M., 1989. — Expression of a novel histone H2B during mouse spermiogenesis. *Developmental Biology*, **133**: 83-92.

71. MOSS, S. B., FERRY, R. A. & GROUDINE, M., 1994. — An alternative pathway of histone mRNA 3' end formation in mouse round spermatids. *Nucleic Acids Research*, **22**: 3160-3166.

72. NAGATA, T., KATO, T., MORITA, T., NOZAKI, M., KUBOTA, H., YAGI, H. & MATSUSHIRO, A., 1991. — Polyadenylated and 3' processed mRNAs are transcribed from the mouse histone H2A.X gene. *Nucleic Acids Research*, **19**: 2441-2447.

73. OLIVA, R. & DIXON, G. H., 1991. — Vertebrate protamine genes and the histone-to protamine replacement reaction. *Progress in Nucleic Acids Research and Molecular Biology*, **40**: 25-94.

74. OSLEY, M. A., 1991. — The regulation of histone synthesis in the cell cycle. *Annual Review of Biochemistry*, **60**: 827-861.

75. OWEN, F. L., TAYLOR, B. J., ZWEIDLER, A. & SEIDMAN, J. G., 1986. — The murine γ-chain of the T cell receptor is closely linked to a spermatocyte specific histone gene and the beige coat color locus on chromosome 13. *The Journal of Immunology*, **137**: 1044-1066.

76. PARSEGHIAN, M. H., HENSCHEN, A. H., KRIEGLSTEIN, K. G. & HAMKALO, B. A., 1994. — A proposal for a coherent mammalian histone H1 nomenclature correlated with amino acid sequences. *Protein Science*, **3**: 575-587.

77. PLATZ, R. D., GRIMES, S. R., MEISTRICH, M. L. & HNILICA, L. S., 1975. — Changes in nuclear proteins of rat testis cells separated by velocity sedimentation. *Journal of Biological Chemistry*, **250**: 5791-5800.

78. RAO, B. J., BRAHMACHARI, S. K. & RAO, M. R. S., 1983. — Structural organization of the meiotic prophase chromatin in the rat testis. *Journal of Biological Chemistry*, **258**: 13478-13485.

79. RASHEED, B. K. A., WHISENANT, E. C., GHAI, R. D., PAPAIOANNOU, V. E. & BHATNAGAR, Y. M., 1989. — Biochemical and immunocytochemical analysis of a histone H1 variant from the mouse testis. *Journal of Cell Science*, **94**: 61-71.

80. RASHEED, B. K. A., WHISENANT, E. C. & BHATNAGAR, Y. M., 1989. — Physical mapping of mouse histone gene clusters. *Biochimica et Biophysica Acta*, **1048**: 110-112.

81. SCHÖLER, H., DRESSLER, G. R., BALLING, R., ROHDEWOHLD, H. & GRUSS, P., 1990. — Oct-4: a germline-specific transcription factor mapping to the mouse t-complex. *EMBO Journal*, **9**: 2185-2195.

82. SCHÜMPERLI, D., 1988. — Multilevel regulation of replication-dependent histone genes. *Trends in Genetics*, **4**: 187-191.

83. SEYEDIN, S. M. & KISTLER, W. S., 1980. — H1 histone subfractions of mammalian testis. *Biochemistry*, **18**: 1371-1375.

84. SEYEDIN, S. M. & KISTLER, W. S., 1980. — Isolation and characterization of rat testis H1t. *Journal of Biological Chemistry*, **255**: 5949-5954.

85. SEYEDIN, S. M. & KISTLER, W. S., 1981. — H1 histones from mammalian testes. H1t is associated with spermatogenesis in humans. *Experimental Cell Research*, **143**: 452-454.

86. SEYEDIN, S. M., COLE, R. D. & KISTLER, W. S., 1981. — H1 histones from mammalian testes. The widespread occurrence of H1t. *Experimental Cell Research*, **136**: 399-405.

87. SHIRES, A., CARPENTER, M. P. & CHALKLEY, R., 1975. — New histones found in mature mammalian testes. *Proceedings of the National Academy of Sciences USA*, **72**: 2714-2718.

88. SITTMAN, D. B., CHIU, I. M., PAN, C. J., COHN, R. H., KEDES, L. H. & MARZLUFF, W. F., 1981. — Isolation of two clusters of mouse histone genes. *Proceedings of the National Academy of Sciences USA*, **78**: 4078-4082.

89. SITTMAN, D. B., GRAVES, R. A. & MARZLUFF, W. F., 1983. — Structure of a cluster of mouse histone genes. *Nucleic Acids Research*, **11**: 6679-6697.

90. THOMAS, J. O. & FURBER, V., 1976. — Yeast chromatin structure. *FEBS Letters*, **66**: 274-280.

91. TROSTLE-WEIGE, P. K., MEISTRICH, M. L., BROCK, W. A. & NISHIOKA, K., 1984. — Isolation and characterization of TH3, a germ cell specific variant of histone 3 in the rat testis. *Journal of Biological Chemistry*, **259**: 8769-8776.

92. TROSTLE-WEIGE, P. K., MEISTRICH, M. L., BROCK, W. A., NISHIOKA, K. & BREMER, J. W., 1982. — Isolation and characterization of TH2A, a germ-cell specific variant of histone 2A in the rat testis. *Journal of Biological Chemistry*, **257**: 5560-5567.

93. VAN HOLDE, K. E., 1989. — *Chromatin*. Berlin, Springer Verlag: 1-497.

94. WOLFE, S. A., ANDERSON, J. V., GRIMES, S. R., STEIN, G. S. & STEIN, J. L., 1989. — Comparison of the structural organization and expression of germinal and somatic rat histone H4 genes. *Biochimica et Biophysica Acta*, **1007**: 140-150.

95. WOLFFE, A. P., 1992. — *Chromatin: Structure and Function*. London, Academic Press: 1-213.

96. WU, R.S. & BONNER, W. M., 1981. — Separation of basal histone synthesis from S-phase histone synthesis in dividing cells. *Cell*, **27**: 321-330.

97. YANG, Y. S., BROWN, D. T., WELLMAN, S. E. & SITTMAN, D. B., 1987. — Isolation and characterization of a mouse fully replication-dependent H1 gene within a genomic cluster of core histone genes. *Journal of Biological Chemistry*., **262**: 17118-17125.

98. YONGVANICH, T. & SVASTI, J., 1984. — Structural differences between somatic H2B and testis-specific TH2B histones of the rat. *Experientia*, **40**: 845-846.

99. ZLATANOVA, J. & DOENECKE, D., 1994. — Histone H1°: a major player in cell differentiation? *FASEB Journal*, **8**: 1260-1268.