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Histones H1 and H4 of surface-spread meiotic chromosomes

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Abstract. The chromatin conformation of somatic and meiotic chromosomes is, at least in part, a function of electrostatic nucleosome interactions that are mediated by transient acetylation of the histone H4 N-terminal domain and phosphorylation of histone H1. The distribution of those histones in the chromatin of meiotic chromosomes is reported here. Antibodies to testis-specific histone 1, H1t, detect H1t in the chromatin of mouse meiotic prophase chromosomes only after synapsis and synaptonemal complex (SC) assembly is completed and before core separation is initiated. The H1t protein is evenly distributed over euchromatin, heterochromatin and the SC. Antibodies to acetylated lysine residues 5, 12 or 16 of histone H4, indicate that the euchromatin is more acetylated than the centromeric heterochromatin. The pattern is most pronounced for acetylated residue 5 and least for 16. Antibodies to phosphorylated H1 epitopes do not react with chromatin but, instead, recognize the chromosome cores and SCs. Possibly these are not phosphorylated histone H1 epitopes, but SC proteins with similar potentially phosphorylatable sequences such as KTPTK of the synaptic protein Syn1.

Introduction

The types and quantities of histones present during spermatogenesis have been reported (Meistrich and Brock 1987; Grimes et al. 1992; Kremer and Kistler 1991) but their distribution in individual meiotic chromosomes is less well documented. With surface-spread testicular cells, the DNA and protein organization of meiotic chromosomes and nuclei can be visualized with fluorescent immuno-cytology and fluorescent in situ hybridization (FISH) (Dobson et al. 1994; Heng et al. 1994). This paper reports the results of histone localizations in meiotic chromosomes and nuclei as demonstrated with well-characterized antibodies to several histones: testis-specific histone H1t (Seyedin and Kistler 1980), acetylated histone H4 (Turner et al. 1989; Jeppesen and Turner 1993), and phosphorylated histone H1 (Lu et al. 1994).

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Materials and methods

The method of preparing surface-spread mouse testicular cells was based on the techniques of Counce and Meyer (1973) with later modifications by Dresser and Moses (1979). These preparations were immunostained as reported previously (Dobson et al. 1994). Fluorescence was protected against fading with ProLong Antifade (Mol. Probes, Eugene, Ore.) mounting agent. The anti-rat histone H1t (testis-specific histone H1) rabbit serum was a gift of W. S. Kistler (University of South Carolina, USA). The rabbit antibodies to acetylated lysine residues 5, 12, or 16 of histone H4 were a gift from B. S. Turner (University of Birmingham, UK). The anti-phosphorylated histone 1 rabbit serum was a gift from C. D. Allis (Syracuse University, USA). The latter serum, prepared against *Tetrahymena* phosphorylated histone 1, recognizes such epitopes in mammalian cells (Lu et al. 1994). Sera were used at dilutions of 1/500 and 1/1000. Preimmune sera tested negative on meiotic chromosomes.

To correlate the occurrence of histones with the presence of cores/SCs (synaptonemal complexes) in the meiotic prophase chromosomes, the cores/SCs were immunostained with mouse or rabbit serum against SC proteins (Dobson et al. 1994) used at a dilution of 1/500, and fluorescein isothiocyanate (FITC-) or rhodamine-conjugated secondary antibodies (Pierce). The DNA was stained with 5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in the mounting medium.

Results and discussion

Testis-specific histone H1t

The histone H1t gene is transcribed and translated first in pachytene primary spermatocytes (Meistrich et al. 1985; Kremer and Kistler 1991; Grimes et al. 1992). The advent and distribution of the histone H1t protein can be monitored in surface-spread pachytene chromosomes. In Fig. 1 the chromosomes of a pachytene spermatocyte nucleus are immunostained with anti-H1t serum and with

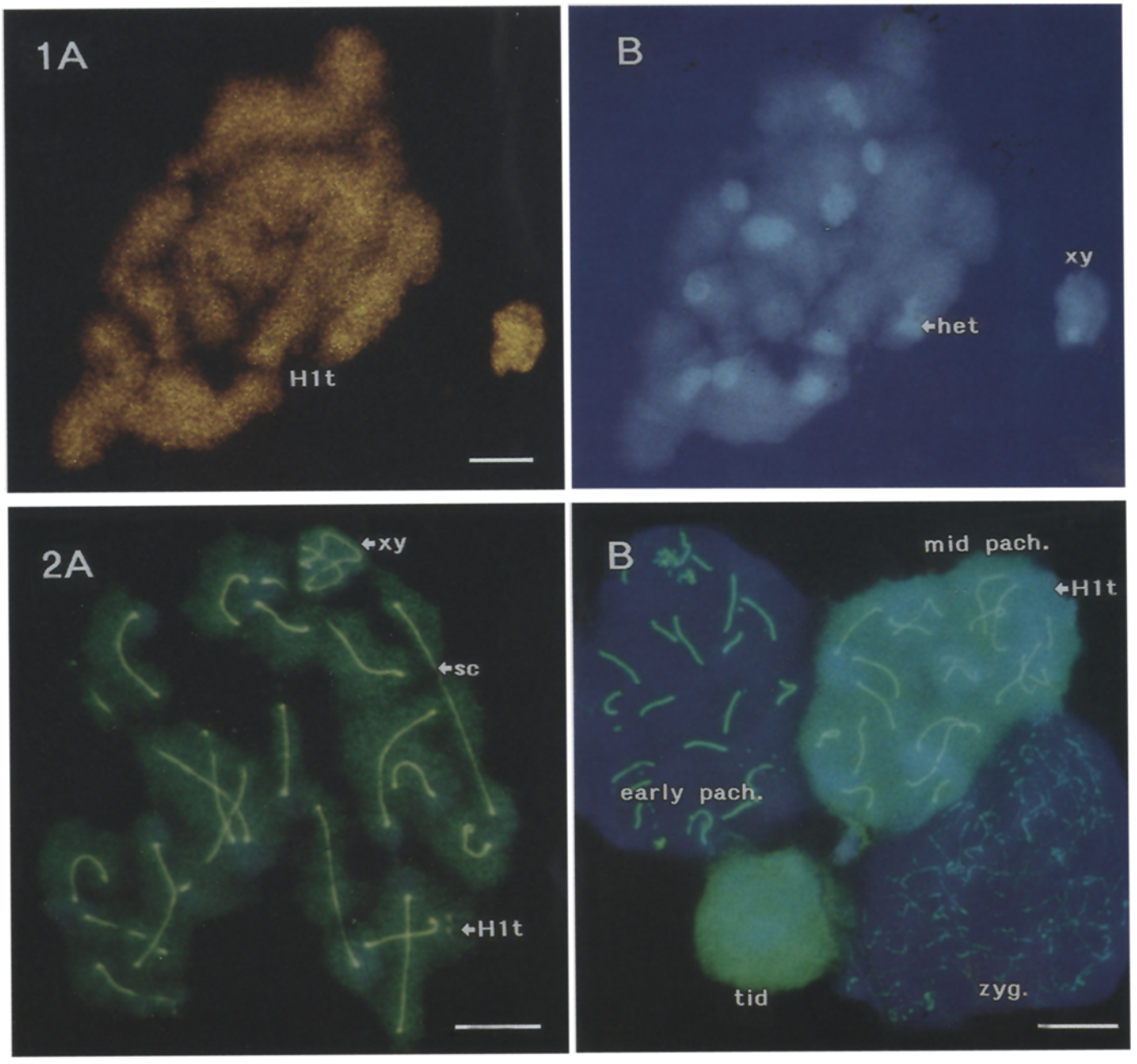
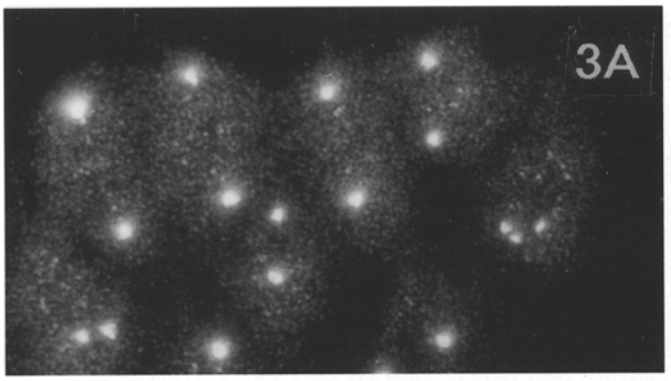
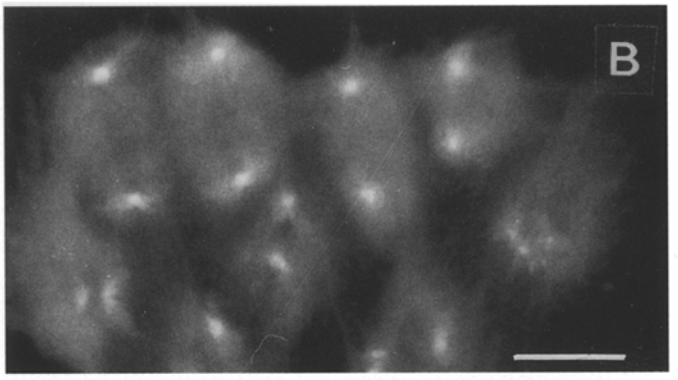
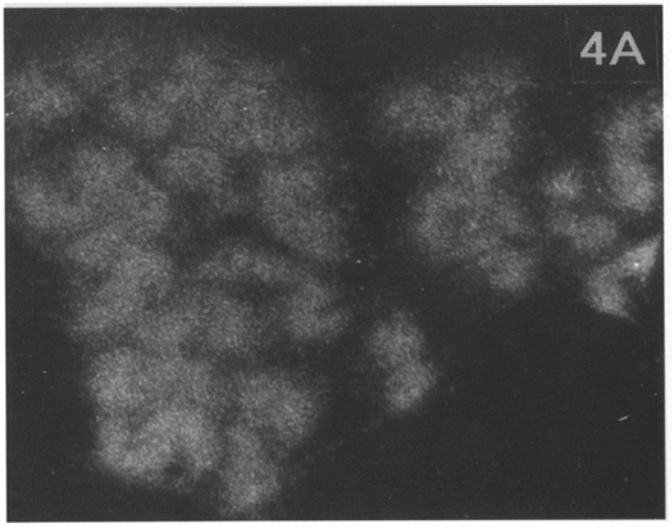


Fig. 1A, B. Indirect immunofluorescence of surface-spread mouse spermatocytes immunostained with antibodies to testis-specific histone, H1t, and counter-stained with the DNA binding dye DAPI (4′,6-diamidino-2-phenylindole) *(blue).* **A** Rhodamine *(red)* fluorescent secondary antibody marks the H1t protein, *H1t,* in association with chromatin of the pachytene chromosomes. **B** *Blue* fluorescent DAPI stain marks the DNA of the same chromosomes. The *bright blue areas* are the centromeric heterochromatin, *het.* The separate structure is the sex vesicle with the X and Y chromosomes, *xy.* Bar represents 10 μm

Fig. 2A, B. Combined immunostaining of histone H1t with anti-H1t antibody and chromosome cores/SCs (synaptonemal complexes) with anti-Cor1 antibody. H1t and cores are fluorescent *green* and the DNA, *blue.* **A** The SC of each bivalent, *sc,* is made visible with antibody to the chromosome core protein Cor1. The chromatin surrounding the SC is immunostained with anti-H1t antibody, *H1t.* The *blue* fluorescent DAPI stain is mostly obscured by the *green* fluorescent FITC (fluorescein isothiocyanate) but is visible at the centromeric heterochromatin. The sex vesicle contains the unpaired cores of the X and Y chromosomes, *xy.* **B** A demonstration of the abrupt appearance of histone H1t during mid-pachytene of meiotic prophase. The zygotene nucleus, *zyg,* is in the process of chromosome synapsis and it does not have any histone H1t. In the early pachytene nucleus, *early pach,* the chromosomes are fully synapsed and there still is no histone H1t. The mid-pachytene nucleus, *mid pach,* is more advanced and it is heavily stained by anti-H1t antibody, *H1t.* Histone H1t is still present in the smaller round spermatid nucleus (*tid*). Bar represents 10 μm







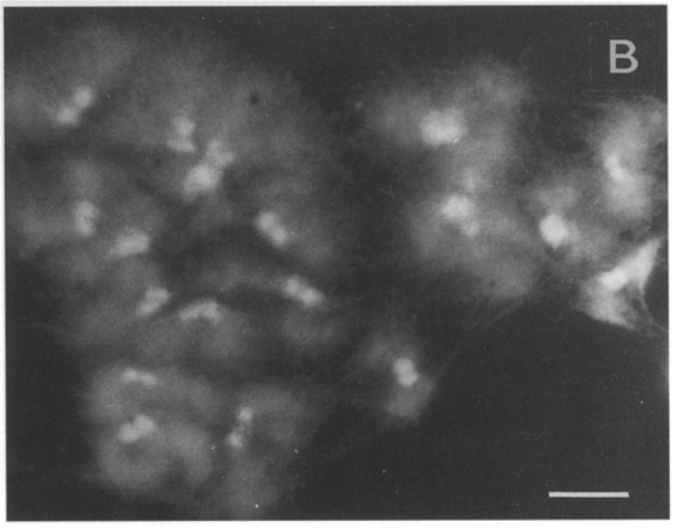


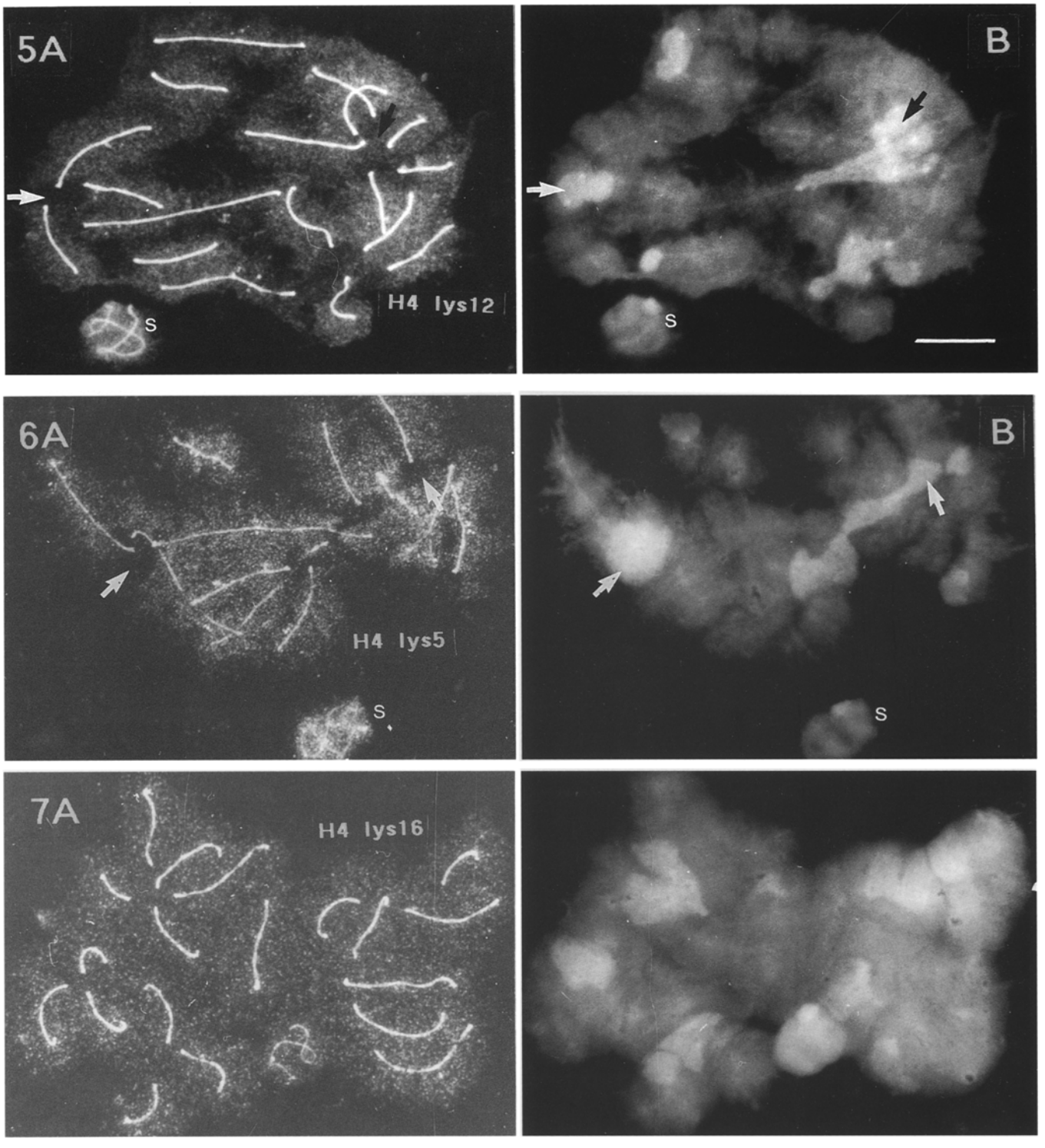
Fig. 3A, B. Metaphase I bivalents. **A** Immune staining with antibody to histone H1t which is diffusely present in the chromatin, and with anti Cor1 which recognizes the centromeric regions at this stage. **B** DAPI staining of the same chromosomes. The *bright dots* are centromeric regions. Bar represents 10 μm

Fig. 4A, B. Metaphase II chromosomes. **A** Immune stained for histone H1t. **B** The same chromosomes DAPI stained. The bright DAPI stain marks the centromeric heterochromatin. Bar represents 10 μm

rhodamine-conjugated secondary antibody (Fig. 1A). The same chromosomes in Fig. 1B are stained with the DNA-binding fluorochrome, DAPI. The brightest DAPI fluorescence is at the centromeric heterochromatin (het) located at one end of each mouse chromosome. There is no corresponding variation in the intensity of H1t fluorescence in Fig. 1A. Similarly, the SCs, to which the chromatin loops are attached and which are visible in Fig. 2A (sc), are not differentiated in terms of H1t intensity in Fig. 1A. The X and Y chromosomes (Fig. 1, xy) of the sex vesicle are of equal intensity to the autosomes. Apparently, the H1t proteins are evenly distributed along the length and width of the pachytene chromosomes independently of the chromatin constitution.

In surface-spread chromosomes the stages of prophase spermatocyte development can be defined by the progression of chromosome alignment, synapsis and disjunction. These characteristics can be monitored precisely with antibodies to the meiotic chromosomes cores (Fig. 2A, Dobson et al. 1994). In Fig. 2, the chromosome core proteins are made visible with anti-core antibody Cor1. It can be seen in Fig. 2B that H1t is not present during the pairing of chromosomes in the zygotene nucleus (zyg.) or the early pachytene nucleus (early pach.) where chromosome synapsis is complete. In the mid-pachytene nucleus (mid. pach.), H1t is plentiful and it is still present in the small nucleus of the round spermatid (tid). The evidence shows that H1t rapidly becomes a part of the chromatin at the mid-pachytene stage of meiosis. This agrees with the observations of Meistrich and Brock (1987) that histone H1t reaches its maximum in spermatocyte nuclei after chromosome pairing is complete.

The presence of histone H1t in the chromatin of metaphase 1 bivalents is shown in Fig. 3. The H1t domains coincide with the DAPI-stained DNA. The centromeric heterochromatin is bright in the DAPI image and the corresponding regions contain bright centromeric regions in the immunostained image because of the accumulation of Cor1 protein at the centromeres (Dobson et al. 1994). When the Cor1 protein is not immunostained, there are no local differences in the H1t density as is demonstrated in Figs. 1 and 4. The distribution of H1t in metaphase II chromosomes is illustrated in Fig. 4. Here, too, there is no differentiation of immunostained chromosomes regions.



Figs. 5, 6, 7. Indirect immunofluorescence of mouse meiotic prophase chromosomes reacted with antibodies to acetylated histone 4 epitopes and with anti-Cor1 antibodies (panels **A**), and counterstained with DAPI (panels **B**). Similar magnifications, bar represents 10 μm

Fig. 5. Antibody to acetylated lysine residue 12. The *arrows* mark the regions of centromeric heterochromatin that appear to be somewhat deficient in acetylated residue 12 relative to euchromatin. The sex vesicle, *s*, has the same fluorescence as the euchromatin

Fig. 6. Residue 5 is similarly under-acetylated in the centromeric heterochromatin *(arrows)*

Fig. 7. Residue 16 seems different in having very little difference between acetylation levels in eu- and heterochromatin

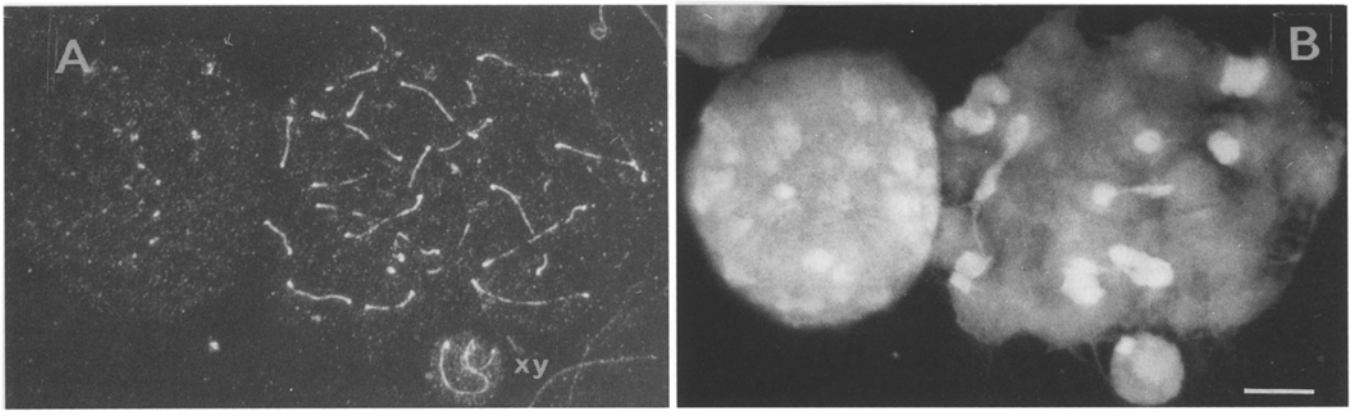


Fig. 8A, B. Anti-phosphorylated histone H1 recognizes chromosome cores/SCs. **A** The single X–Y, *xy,* cores are labelled as well as the SCs. There is only slight labelling of the chromatin. **B** The corresponding DAPI-stained nucleus. Bar represents 10 μm

Acetylated histone H4 residues 5, 12 and 16

Acetylation of histone H4 is generally associated with transcriptionally active chromatin. Jeppesen and Turner (1993) report that chromosomal R-bands, which are enriched in coding DNA, have acetylated histone H4 but the inactive X chromosome in mammalian female cells lacks the acetylated H4 (reviewed by Loidl 1994).

The distribution of acetylated H4 in mouse spermatocyte meiotic chromosomes follows the general pattern but with some qualifications. The absence of acetylated H4 in the centromeric heterochromatin is most pronounced for antibodies to acetylated lysine residue 5 (Fig. 6), quite clear for lysine residue 12 (Fig. 5), but less obvious for residue 16 (Fig. 7). For each residue, panels A show the immune-labelling pattern while panels B demonstrate the DAPI-stained pachytene chromosomes of the same nucleus. These observations indicate that there is a process of differential H4 acetylation in the constitutive heterochromatin of meiotic chromosomes. A difference with the labelling pattern of mitotic chromosomes is the apparent absence of a banding pattern. Possibly the surface-spreading procedure preserves the chromatin loop structure so that local differences in immunostaining densities are too diffuse to observe.

Phosphorylated histone H1

Surprisingly, the antibodies to phosphorylated histone 1 have a strong preference for the meiotic chromosome cores and less so for the chromatin (Fig. 8). D. Allis and co-workers have shown that, although histone H1 differs between *Tetrahymena* and mammals, the phosphorylation site is conserved and the antibodies raised against the *Tetrahymena* protein cross-react in a cell cycle-dependent manner with phosphorylated histone H1 of HeLa cells (Lu et al. 1994). It seems unlikely, however, that there is a concentration of phosphorylated histone H1 in the chromosome cores. Instead, the SC proteins may have epitopes that mimic the phosphorylated histone 1 site. A perfect match to the consensus sequence K/R-S/T-P-X-K/R exists in the synaptic protein, Syn 1, K-T-P-T-K (Meuwissen et al. 1992; Moens et al. 1992, accession number L32978). Several other sites have lesser similarity. The fact that unpaired cores of the X and Y chromosomes (Fig. 8) and the unpaired cores of the diplotene stage are also recognized by the antibody suggests that not only the synaptic protein but also the core proteins may have similar epitopes. The Cor1 protein has, at best, 70% matches with the consensus sequence. This may be sufficient for epitope recognition or there may be other core proteins with better matches. To check the assumption that alternative proteins can react with the serum, it was tested on sporulation yeast *(Saccharomyces cerevisiae),* which lacks histone H1. The small fraction of cells that gave a strong positive reaction give support to the notion that phosphorylated proteins other than histone H1 may be recognized by the serum.

Conclusions

Post-translational modifications that alter the electrostatic charges of histones H1 and H4 have been implicated in transcriptional processes and in chromosome condensation and decondensation (reviews by Roth and Allis 1992; Loidl 1994), but the actual mechanisms are not well resolved. Levels of acetylated histone H4 and testis-specific histone H1 remain high throughout meiotic prophase, into metaphase I and in later stages. Apparently, cell cycle dependence is lacking for those histones or for their modifications at meiosis. In surface-spread chromosomes, the chromatin loops are relaxed by the use of a surface surfactant during drying. In such preparations, it appears that the transition from prophase to metaphase I chromosomes is accomplished not so much by chromatin condensation through shortening of the loops, but rather by a shortening of the chromosome axis and consequent compacting of the loops. Possibly, meiotic chromatin does not require the traditional cell cycle-dependent regulation of transcription and condensation that is observed in somatic cells.

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