Structurally Divergent Histone H1 Variants in Chromosomes Containing Highly Condensed Interphase Chromatin

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Abstract. Condensed and late-replicating interphase chromatin in the Dipertan insect *Chironomus* contains a divergent type of histone H1 with an inserted KAP-KAP repeat that is conserved in single H1 variants of *Caenorhabditis elegans* and *Volvox carteri*. H1 peptides comprising the insertion interact specifically with DNA. The Chironomid *Glyptotendipes* exhibits a corresponding correlation between the presence of condensed chromosome sections and the appearance of a divergent H1 subtype. The centromere regions and other sections of *Glyptotendipes barbipes* chromosomes are inaccessible to immunodecoration by anti-H2B and anti-H1 antibodies one of which is known to recognize nine different epitopes in all domains of the H1 molecule. Microelectrophoresis of the histones from manually isolated unfixed centromeres revealed the presence of H1 and core histones. H1 genes of *G. barpipes* were sequenced and found to belong to two groups. H1 II and H1 III are rather similar but differ remarkably from H1 I. About 30% of the deduced amino acid residues were found to be unique to H1 I. Most conspicuous is the insertion, SPAKSPGR, in H1 I that is lacking in H1 II and H1 III and at its position gives rise to the sequence repeat SPAKSP-AKSPGR. The homologous H1 I gene in *Glyptotendipes salinus* encodes the very similar repeat TPA-KSPAKSPGR. Both sequences are structurally related to the KAPKAP repeat in H1 I-1 specific for condensed chromosome sites in *Chironomus* and to the SPKKSPKK repeat in sea urchin sperm H1, lie at almost the same distance from the central globular domain, and could interact with linker DNA in packaging condensed chromatin.

DIFFERENT linker histones can be differentially distributed within chromosomes. In the midge, *Chironomus thummi*, the centromere regions and a number of other chromosome bands contain a specific sequence variant of H1, H1 I-1, that cannot be detected in the majority of sites in the polytene chromosomes (Mohr et al., 1989). H1 I-1 contains a novel DNA-binding motif that is lacking in the other H1 histones of *C. thummi* but is evolutionarily conserved in one of the H1 histones of the Nematode worm, *Caenorhabditis elegans*, and of the green alga, *Volvox carteri* (Schulze et al., 1993). H1 I-1 is more abundant in the chromatin of the subspecies *C. th. thummi* than in that of *C. thummi piger*, a difference also evident from a number of homologous chromosome bands in both subspecies that are immunodecorated by H1 I-1–specific antibodies in *C. th. thummi* but exhibit no immunofluorescence in *C. th. piger* (Mohr et al., 1989). Many of the *C. th. thummi* chromosome loci containing H1 I-1 differ from their homologous counterparts in the *piger* genome also in that they replicate late in S-phase (Keyl and Pelling, 1963), stain in a C-banding procedure (Hägele, 1977), and contain repeats of specific satellite DNA sequences (Schmidt, 1984). We have therefore proposed that the DNA binding motif inserted in the NH_2-terminal domain of H1 I-1 may interact with linker DNA and may be involved in establishing a specifically condensed subtype of chromatin (Schulze et al., 1993).

While chromatin subtypes with different packaging in interphase would be difficult to detect in most organisms, some insect species exhibit polytene chromosome structures that appear differentially condensed. Among these, the extended centromere regions in the Chironomid genus *Glyptotendipes* are especially conspicuous and have aroused the interest of cytologists for many years. In the polytene chromosomes they usually appear as prominent blocks of condensed chromatin. Occasionally their structure is loosened and becomes a puff-like local decondensation, but this dramatic change in appearance is not accompanied by any noticeable DNA synthesis such as is known for the "DNA-puffs" in Sciarids (Crouse and Keyl, 1968; Walter, 1973). The conspicuous morphological differences between centromere and other chromosome regions in *Glyptotendipes* presumably mirror differences in chromatin structure. We report here that the centromere regions and a number of other chromosome sections, in contrast to the majority of chromosome sites, are not decorated by antibodies against histone H1 and histone

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H2B even after extensive decondensation. The presence of H1 and core histones was demonstrated by microelectrophoresis in capillaries of extracts from centromeres that were manually isolated from unfixed chromosomes. Therefore these structures must have a specific molecular architecture that renders histones inaccessible to antibodies.

In an approach to elucidate properties of the chromatin architecture in centromere regions, we have asked whether *Glyptotendipes* contains a divergent histone H1 of the type specific for condensed interphase chromatin in *Chironomus* (Mohr et al., 1989). We find that *Glyptotendipes*, like *Chironomus*, contains two classes of H1 genes. One of the H1 gene types in both *G. barbipes* and *Glyptotendipes salinus* differs from the other H1 genes by an insertion that in the NH_2-terminal domain creates the protein motif, SPAKSPAKSPGR, in *G. barbipes* and TPAKSPAKSPGR in *G. salinus*. Both structures are similar to those of the KAPKAP motif in *Chironomus* H1 I-1 and the SPKKSPKK motif specific for sea urchin sperm H1. It is tempting to speculate that they are involved in establishing the condensed chromatin in the centromere and other regions.

Materials and Methods

Antibodies

The elicitation of mouse monoclonal antibodies directed against histone H1 of *C. thummi* has been described earlier (Mohr et al., 1989). The polyclonal rabbit antibody against *C. thummi* H1 was also raised in our laboratory (Mohr, 1984; Westermann and Grossbach, 1984). The polyclonal rabbit antibody directed against histone H2B was the generous gift of Dr. Martin Blumenfeld (University of Minnesota, St. Paul, MN). The monoclonal mouse antibody against Drosophila H2A was the generous gift of Dr. H. Saumweber (Humboldt University, Berlin, Germany). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG and goat anti-rabbit IgG were purchased from Medag (Hamburg, Germany).

Chromosome Squash Preparations and Indirect Immunofluorescence

Salivary glands were dissected from last instar larvae in Robert's Ringer solution (Robert, 1975). For staining in orcein-carmin 1:1 (0.5% in 50% acetic acid) they were fixed for 3 min in ethanol-acetic acid (3:1). After staining for 1 h, the cells were isolated in 50% acetic acid, squashed, frozen in dry ice, and embedded in Euparal. For antibody decoration the glands were fixed in 0.5% formaldehyde and treated as described earlier (Westermann and Grossbach, 1984), except that Robert's Ringer was replaced by Tris-buffered saline (0.15 M NaCl, 0.02% sodium azide, 10 mM Tris, pH 7.2) for the antibody solutions and the washing steps. The fluorescent dye-conjugated second antibodies were affinity-purified before use with formaldehyde-fixed and homogenized Chironomus salivary glands as described (Westermann and Grossbach, 1984). The IgG fraction of the polyclonal anti-H1 antibody was purified according to Axelson et al. (1973) and used at a dilution of 1:20. The monoclonal antibody from clone 4/H9 of our series of clones producing antibodies against histone H1 of *C. thummi* (Mohr, 1984; Mohr et al., 1989) was used at a dilution of 1:1000. The anti-H2B antibody was applied at dilutions of 1:30 and 1:60. After incubation with the second antibody, the preparations were stained for DNA in 4 μM of the fluorescent dye Hoechst 33342, washed repeatedly in Tris-buffered saline, and mounted in Tris-buffered saline-glycerol (1:9) containing 0.1% *p*-phenylenediamine. Photomicrographs were made on Kodak Tri X Pan film through a Zeiss fluorescence microscope.

Isolation of Centromere Regions from Unfixed Chromosomes

Salivary glands of last instar larvae were dissected in Robert's Ringer solution (Robert, 1975) and incubated in the Ringer solution containing 0.32% Triton X-100 and 0.53% Nonidet P-40 for 8–12 min. After this treatment the nuclei could be manually isolated from the gland cells and were transferred into Ringer solution containing 0.03% Triton X-100 and 0.05% Nonidet P-40. The following steps were performed at 4°C, as low temperature was found to be crucial for an efficient isolation of *Glyptotendipes* chromosomes. By means of a 2-ml syringe, the suspension of nuclei was forced through a glass capillary with a tapered tip of 60-μm inner diam. The shearing force exerted on the nuclei by this procedure was found appropriate to yield a high percentage of intact isolated chromosomes from *Glyptotendipes* glands. Centrifugation of the suspension at 50 g in a cylindrical chamber was used to attach the chromosomes to a cover-slip. The chromosomes were then squashed under a second cover-slip and frozen in liquid nitrogen. After removal of the second cover-slip the preparations were stored in liquid nitrogen. The chromosomes were freeze-dried at −65°C in a device for cytological freeze-drying (GT001, Leyboldt-Heraeus, Cologne, Germany), that was modified to higher efficiency, and stored in vacuo. Centromere regions were isolated from the dry chromosomes on an inverse microscope by means of a glass rod with a tapered tip made on the de Fonbrune microforge (Beaudouin, Paris, France). The glass rod was directed by a de Fonbrune pneumatic micromanipulator and placed at an angle of 45°C to the cover-slip carrying the chromosomes. The chromosomes usually broke at the boundaries of the centromere regions under the slight lateral force of the glass instrument, making it an easy task to isolate the centromere regions. These were collected in a droplet of a few nanoliters of electrophoresis buffer protected by paraffin oil in an oil chamber as described by Edström (1964). In another procedure, the isolated centromere regions were collected dry by transferring them with the glass rod under microscopic control to a glass fiber of 10-μm-diam, that was attached to the microscope stage and had been made sticky by glue from Scotch Double Stick Tape (3M Consumer Products, St. Paul, MN) dissolved in petrol. About 50–100 centromere regions from chromosomes I, II, and III of *G. barbipes* were collected for every electrophoresis.

Microelectrophoresis in Capillary Gels

The separation of proteins on the nanogram scale in disc-electrophoresis columns of 100-μm-diam was performed as described (Grossbach, 1965; Grossbach and Kasch, 1977) with the following modifications. Separation gel and stacking gel solution (Laemmli, 1970) contained 20% sucrose and acrylamide concentrations of 20 and 10%, respectively. For the transfer of nanoliter droplets of protein sample from the paraffin oil chamber into the capillary column, the oil chamber was fixed to the micromanipulator carrying the micropipette, the sample was sucked into the micropipette, delivered into the capillary on top of the stacking gel, and overlayed with electrode buffer (Laemmli, 1970). Isolated chromosome segments fixed to a glass fiber were transferred by immersing the fiber into the upper part of the capillary column that had been filled with sample buffer. The end of the glass fiber was then cut and the capillary was placed for 15 min into a moist chamber to allow for dissolution of the proteins. Subsequently the glass fiber was removed and the top of the capillary was filled with electrode buffer. Electrophoresis was performed at 0.2 μA per capillary for 2 min, followed by another 6 min at 2.5 μA per capillary. Gels were removed from the capillaries by tightly fitting tungsten wire, and the proteins were fixed for 30 min in 20% trichloroacetic acid, stained in Coomassie blue R250, and photographed through an inverted Zeiss microscope on Agfa T-Max 400 film. Silver staining procedures for proteins (Wray et al., 1981; Merril and Pratt, 1986) that greatly increase the sensitivity on conventional gels were tried with the capillary gels and found to be less sensitive than the Coomassie dye.

Isolation and Sequencing of Histone H1 Genes

Genomic DNA was isolated from 260 mg of larvae of *G. barbipes*, and from one single larva of *G. salinus*, with the procedure described by McGinnis et al. (1983). Histone H1 gene sequences were amplified by the PCR with primer oligonucleotides that were synthesized according to sequences in the 5′ and 3′ regions of *Chironomus* H1 genes. The oligonucleotides GAGGCTTCGTTTCCAT and ATTT(G,T)GTAGTCCTGAAAAGGACT presumably primed the amplification of all H1 genes sharing these sequences that were found to be common to all types of *C. thummi* H1 genes (B. Schulze, 1992; E. Schulze, 1992; Trieschmann, 1992). In contrast, oligonucleotide TTGGTAGTCCTGAAAAGGACTGA contains two bases (*underlined*) specific for the 3′ sequence of the structurally divergent H1 I 1 gene of *C. thummi* and its homologue H1e in *C. tentans* and should thus selectively prime amplification of H1 genes sharing this structure. The strategy for specific PCR proved to be successful in *Glyptotendipes* and resulted in the identification of a structurally divergent H1 gene in both *G. barbipes* and *G. salinus* (see Results). The reaction (Saiki et al., 1988) was

performed for 45 cycles (annealing temperature 46°C) in a Trio-Thermoblock (Biometra, Göttingen, Germany). The amplified DNA fragments were made blunt-ended with T4 DNA polymerase or Taq DNA polymerase and subjected to gel electrophoresis on low melting agarose. The gel slices were transferred into Eppendorf tubes, and the DNA was ligated into the Sma I site of the pUC 18 plasmid (Yanish-Perron et al., 1985) as described by Sambrook et al. (1989). *Escherichia coli* JM103 cells were made competent with $CaCl_2$ and transformed following standard procedures. The sequencing was performed according to Sanger et al. (1977) with a DNA sequencing kit (version 2.0; USB, Cleveland, OH). In addition to the direct-20 and reverse pUC 18 primers (Stratagene, Heidelberg, Germany), an oligonucleotide, CAATACAA(A,G)GTTGAT(A,G)(C,T)TGA, was constructed for priming that represents an evolutionarily conserved H1 gene sequence encoding a section of the central domain.

Results and Discussion

The Extended Centromere Regions of Glyptotendipes Chromosomes Are Not Decorated by Antibodies against Histones H1 and H2B

The four chromosomes of the *G. barbipes* set contain prominent and extended, highly condensed centromere regions that include several bands (Bauer, 1936). They are in a metacentric position in three of the chromosomes and telocentric in the small chromosome IV (Fig. 1). DNA staining by Hoechst 33342 revealed a very high apparent concentration of DNA in these structures (Fig. 2 *A*). In contrast, indirect immunofluorescence with a polyclonal antibody against histone H1 left the centromere regions completely dark (Fig. 2 *B*). Corresponding results were obtained with a polyclonal antibody directed against histone H2B (Fig. 3) and a monoclonal anti-H2A antibody (not shown). On the other hand, many sections on all chromosome arms were recognized by the antibodies (Figs. 2 *B* and 3). In order to check whether the failing in detecting histones was due to a high-order packaging of chromatin, the centromere regions were decondensed until they reached a puff-like structure. Such centromere "puffs" occur spontaneously sometimes and can also be induced in vivo by exposure to low temperature and by x-ray irradiation (Walter, 1973). Last instar larvae were irradiated at 10.000 r and were 24 h later found to exhibit extensive centromere decondensation (Fig. 4). This process presumably includes loosening of the lateral contact of chromatids as well as some degree of longitudinal unpackaging of chromatin. Centromere regions after decondensation showed lower apparent DNA concentration (Fig. 4 *B*) but remained completely dark after immunofluorescence decoration with anti-H1 antibody (Fig. 4 *C*). A clear border was seen between the dark centromere region and the adjacent chromosome sites that were recognized by the antibody (Fig. 4, *C* and *D*).

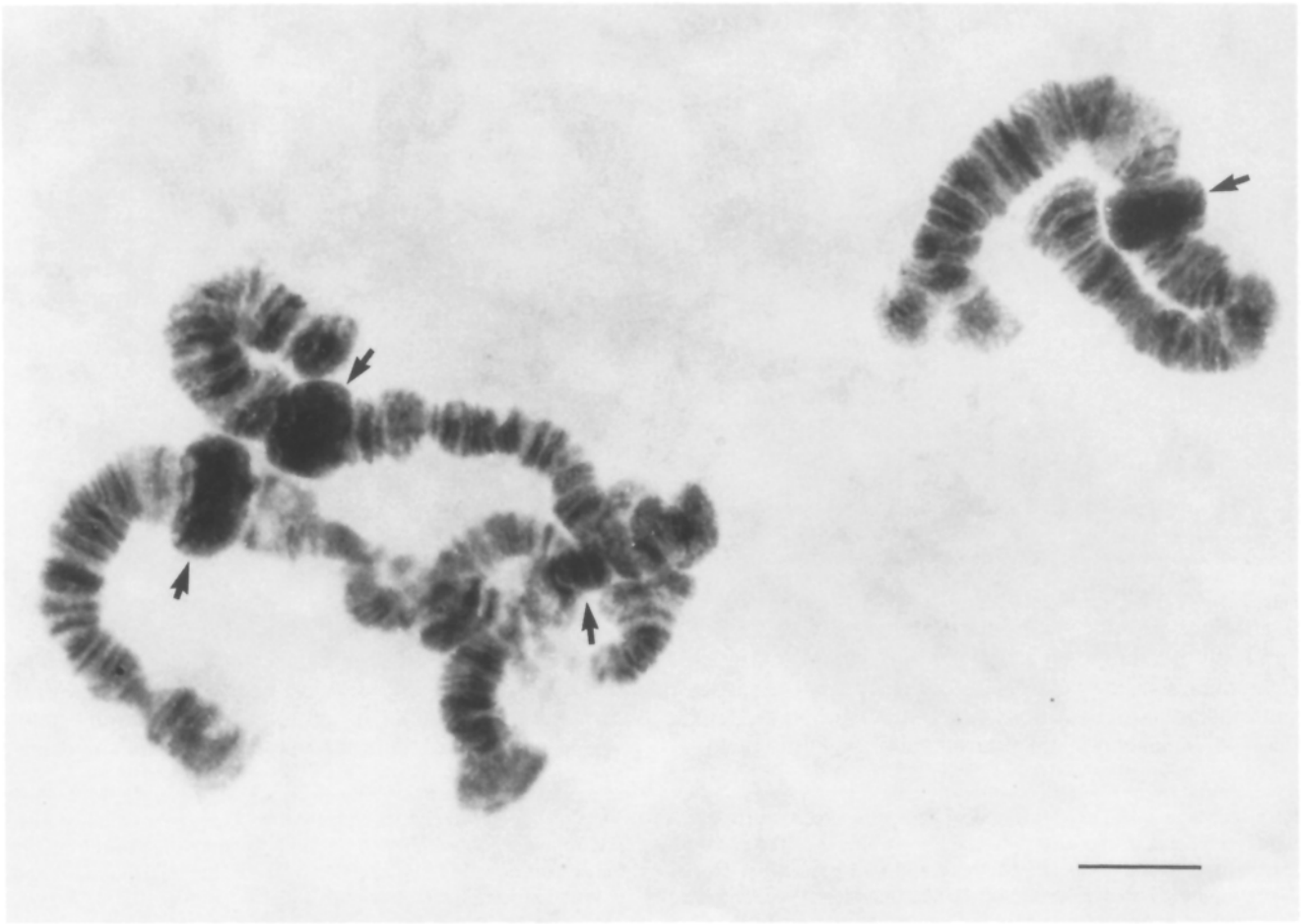


Figure 1. Squash preparation of the set of salivary gland chromosomes of *G. barbipes*. The glands were fixed in ethanol-acetic acid 1:3 and stained in 0.5% carmin, 0.5% orcein in 50% acetic acid. Bright field illumination. The arrows indicate the centromere regions. Bar, 20 μm.

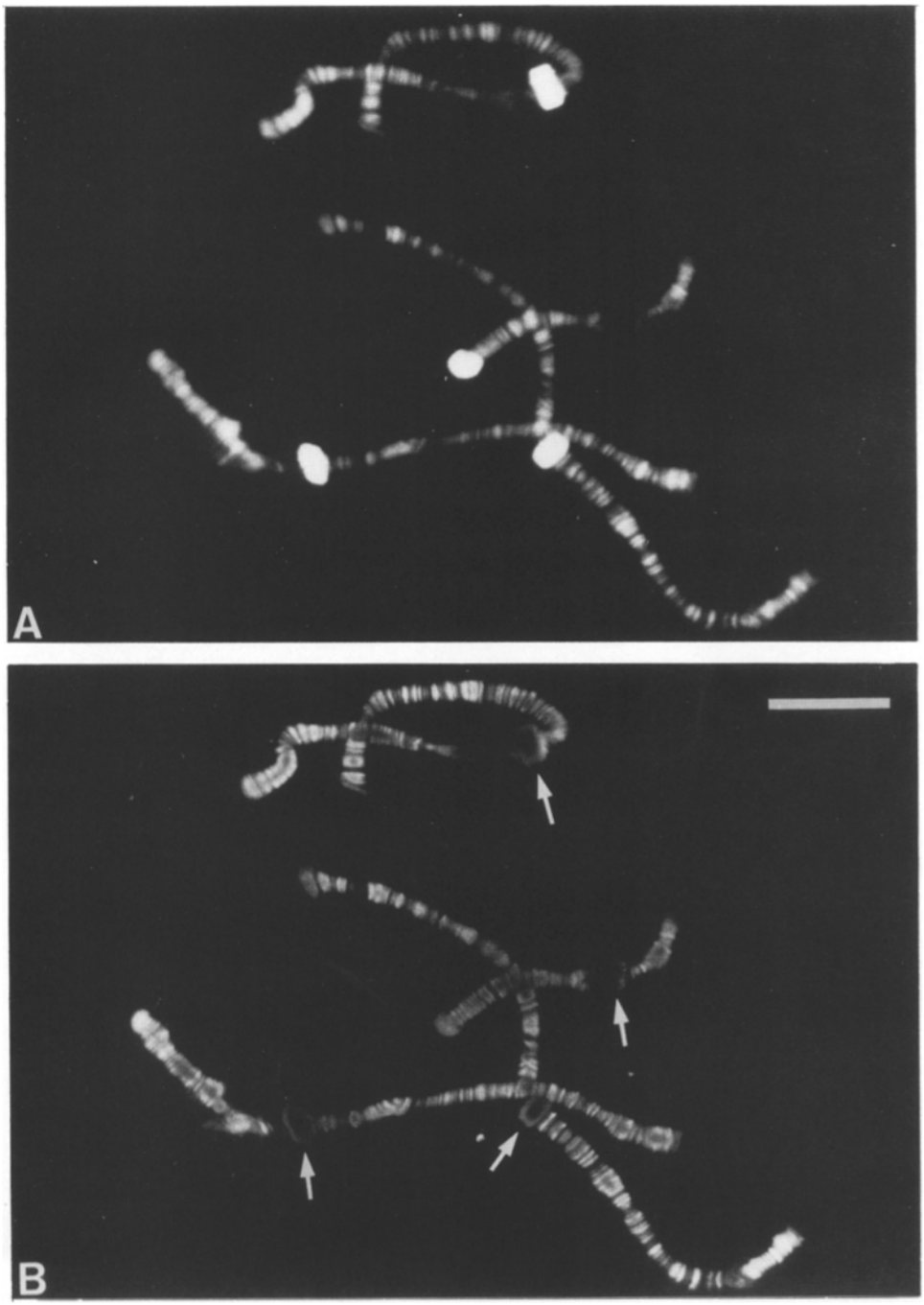


Figure 2. Set of salivary gland chromosomes of *G. barbipes* stained for DNA with Hoechst 33258 (*A*) and immunodecorated with a polyclonal anti-histone H1 antibody (*B*). The glands were fixed in 0.5% formaldehyde, and the cells were dissected in 50% acetic acid, squashed, and frozen on dry ice. They were incubated with antibody for 1 h at 37°C and after washing exposed to fluorescein-isothio cyanate-conjugated goat anti-rabbit immunoglobulin for another hour at 37°C. Note that the centromere regions exhibit a very high apparent DNA content but are not recognized by the antibody (*arrows*). Bar, 50 μm.

We conclude that the observed lack in antibody decoration is probably not due to an especially tight lateral packaging of chromatids in the centromere regions but rather arises from a different organization of the chromatin on the level of the individual chromatid. Chromatin fibers in the centromere regions could be organized in a way that renders H1, H2B, and H2A less accessible than usual, or the chromatin could be devoid of these histones. In addition to the centromere regions, a number of bands within the chromosome arms also remained dark in immunodecoration by anti-

Figure 4. Metacentric chromosome in which the centromere region (*arrows*) has been largely decondensed by X-ray irradiation in vivo to analyze effects of the loss of tight lateral contact of the chromatids on chromatin accessibility to antibody binding. Technical details are described in Materials and Methods and in the legend of Fig. 2. (*A*) Phase contrast; (*B*) fluorescence image of the distribution of the DNA staining dye Hoechst 33342; (*C*) immunofluorescence image of the distribution of a monoclonal anti-H1 antibody (clone 4/H9); (*D*) immunofluorescence image of the same chromosome from another nucleus decorated with the same antibody. Note that the centromere region exhibits a more open structure (*A*) and less intense DNA-staining (*B*) than in Figs. 2 and 3 but remained completely dark in indirect immunofluorescence (*C* and *D*). The decondensed structure to the left (*A–C*) and to the right (*D*) of the centromere is a nucleolus organizer. Bar, 20 μm.

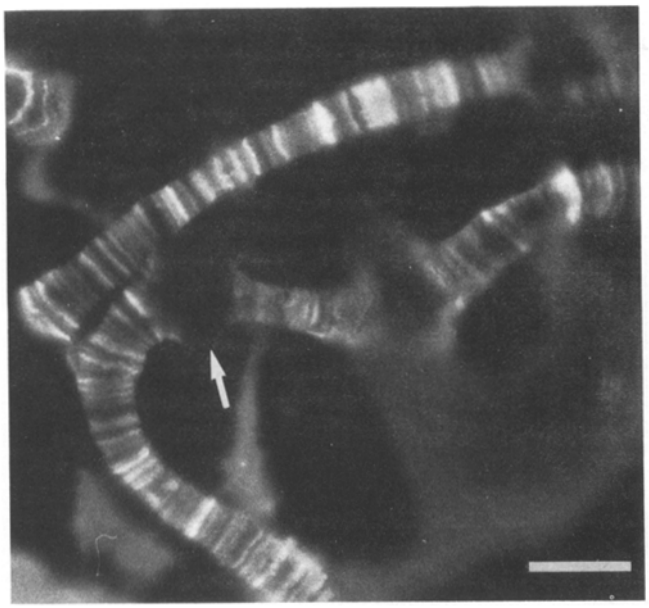
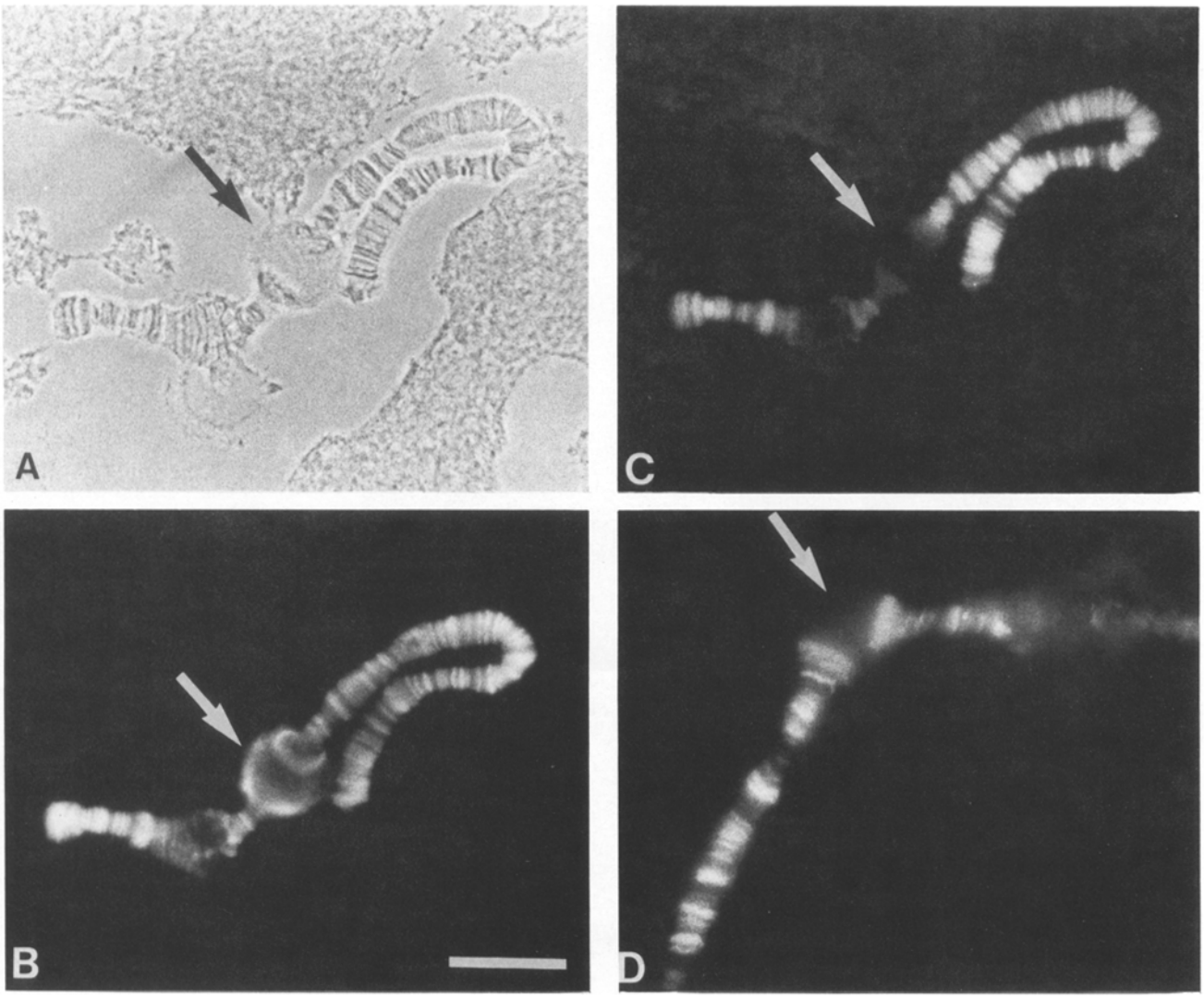


Figure 3. Immunofluorescent image of part of two metacentric salivary gland chromosomes decorated with a polyclonal antibody against histone H2B. Technical details are described in Materials and Methods and in the legend of Fig. 2. The centromere region (*arrow*) and a number of chromosome bands are not decorated by the antibody. Bar, 20 μm.

histone antibodies (compare Figs. 4, *B* and *C*; cf. the dark bands in Fig. 3). These bands may contain chromatin with a similarly divergent structure.

Centromere Regions Contain H1 and Core Histone(s) That Are Inaccessible to Antibodies

Whether a protein is inaccessible or lacking in a cellular structure can be investigated by direct analysis only. We have therefore manually isolated centromere regions from *G. barbipes* salivary gland chromosomes and have separated the histones by microelectrophoresis in capillaries, using a method that has been described earlier (Grossbach, 1965, Grossbach and Kasch, 1973) but was substantially modified for this purpose by E. Schulze (see Materials and Methods). In order to avoid loss or redistribution of proteins, the chromosomes were prepared without fixation. Nuclei were manually isolated from salivary glands explanted in *Chironomus* Ringer solution (Robert, 1975) containing 0.32% Triton



X-100 and 0.53% Nonidet P-40. They were then transferred into Ringer containing a ten times lower concentration of the detergents, and the chromosomes were isolated by forcing the nuclei in solution through a capillary with an opening of 60 μm (for details see Materials and Methods). After transfer onto a cover-slip the chromosomes were squashed, frozen in liquid nitrogen, freeze-dried under conditions suitable for cytological freeze-drying, and stored in vacuo. After this procedure, the centromere regions exhibited a consistency that was different from that of the adjacent chromosome sections and that made it an easy task to separate them with a glass instrument directed by a de Fonbrune micromanipulator. The isolated centromere regions were either transferred into a nanoliter droplet of concentration gel buffer solution under oil or glued onto a 10-μm-diam glass fiber. Electrophoresis was performed on Laemmli gels (Laemmli, 1970) in capillaries of 100-μm-diam. Between 50 and 100 isolated centromere regions per gel from chromosomes I, II, and III were dissolved in concentration gel solution or were directly transferred on the glass fiber into the buffer-filled upper section of an electrophoresis capillary.

The results (Fig. 5) showed that the centromere regions contain H1 as well as core histones. We conclude that histones H1 and H2B in the centromere regions, in contrast to other sections of the chromosomes, are inaccessible to antibodies. The epitope recognized by the monoclonal anti-H1 antibody of clone 4/H9 used in these experiments (Fig. 4) maps near the N-terminus of the H1 molecule (A. Steuernagel, unpublished observation). The epitopes recognized by our polyclonal anti-H1 antibody (Fig. 2) on the H1 molecule have been mapped by using overlapping peptides of 10-amino acid residues length that were synthesized on spots on a membrane and that cover the entire sequence of the protein. Nine different epitopes of the antibody were identified by an immunoreaction on the membrane and were found to be spread in the NH_2-terminal, central globular, and COOH-terminal domains of the H1 molecule (A. Steuernagel, unpublished observation). This strongly indicates that the molecular architecture of the centromere regions differs from that of other chromosome sections in a way that renders the H1 molecules completely inaccessible. Possibly, the chromatin fiber is coated by other protein(s) in a way that makes antibody binding impossible. Alternatively, the centromere regions could contain unusual histone modifications or variants that establish a divergent chromatin structure. The analysis of H1 genes in *Glyptotendipes* has actually revealed a novel H1 variant with a sequence motif that is similar to a motif characteristic of H1 in condensed chromatin in sea urchin sperm (see below).

Nonchev et al. (1989) have earlier provided evidence suggesting the presence of H1 in the centromeres of *Glyptotendipes* chromosomes fixed in acetic acid. As redistribution of proteins between cellular structures during fixation is a frequent phenomenon, we chose to analyze unfixed chromosomes after freeze-drying.

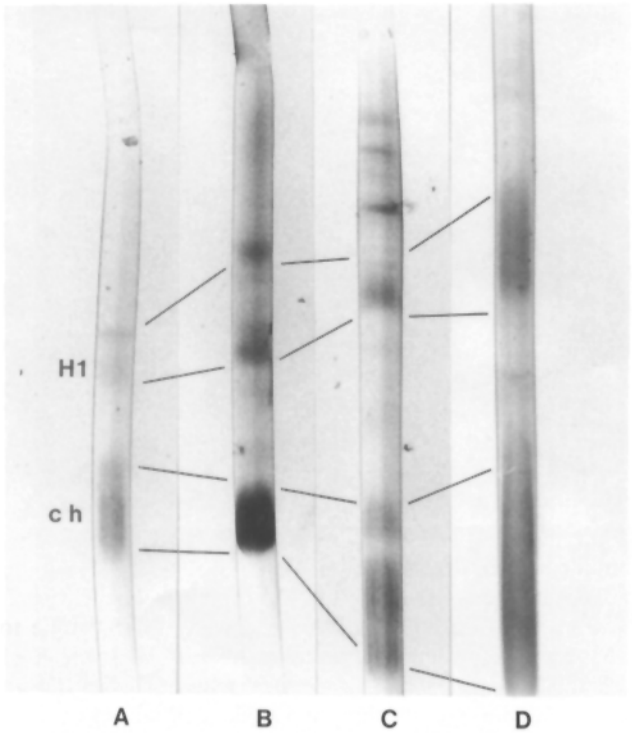


Figure 5. Microelectrophoresis in capillaries (0.1-mm-diam) of histones from 60 centromere regions that had been manually isolated from unfixed chromosomes (*A*); of extracts from three isolated gland nuclei of *Chironomus thummi* (*B* and *C*); and of total histone of *Glyptotendipes* larvae prepared on a conventional scale (*D*). SDS-gels (Laemmli, 1970) were prepared in capillaries of 100-μm-diam by means of a micromanipulator. Gels *B–D* show the degree of histone separation achieved. The individual core histones and the H1 subfractions observed on conventional SDS-gels in *C. thummi* (three) and *G. barbipes* (two) could not be separated on this level. H1: histone(s) H1; *c h*, core histones.

Divergent H1 Subtypes of G. barbipes and G. salinus Contain Motifs Similar to the SPKK Motif in Sea Urchin Sperm H1

Centromeres and other condensed and late-replicating chromosome sites of *Chironomus thummi* contain a structurally divergent subtype of H1 that cannot be detected in the majority of chromosome bands (Mohr et al., 1989). This H1 variant comprises an inserted sequence repeat that is evolutionarily conserved in plants and animals and that possibly interacts with DNA in a specific way (Schulze et al., 1993). We have therefore asked whether the Chironomid *Glyptotendipes* with its large centromere regions contains a correspondingly divergent subtype of histone H1 that could be involved in establishing an especially condensed chromatin structure. Electrophoresis in acetic acid-urea gels in the presence of Triton X-100 revealed two fractions of H1 in *G. barbipes* (Hoyer-Fender and Grossbach, 1988). However, on capillary gels we were not able to discriminate between different H1 subtypes (Fig. 5).

For a comparative analysis of H1 histones in *Glyptotendipes*, H1 genes of *G. barbipes* and *G. salinus* were amplified from genomic DNA by PCR. A strategy was used that exploited sequence differences in the 3′ flanking region between the two types of H1 genes in the genus *Chironomus*. To amplify specifically H1 genes that share sequence peculiarities of the H1 I-1 gene type, a primer oligonucleotide was used that comprises the 3′ flanking hairpin-loop of H1 genes (Birnstiel et al., 1985) preceded by a short sequence that is unique to the divergent *Chironomus* H1 variant

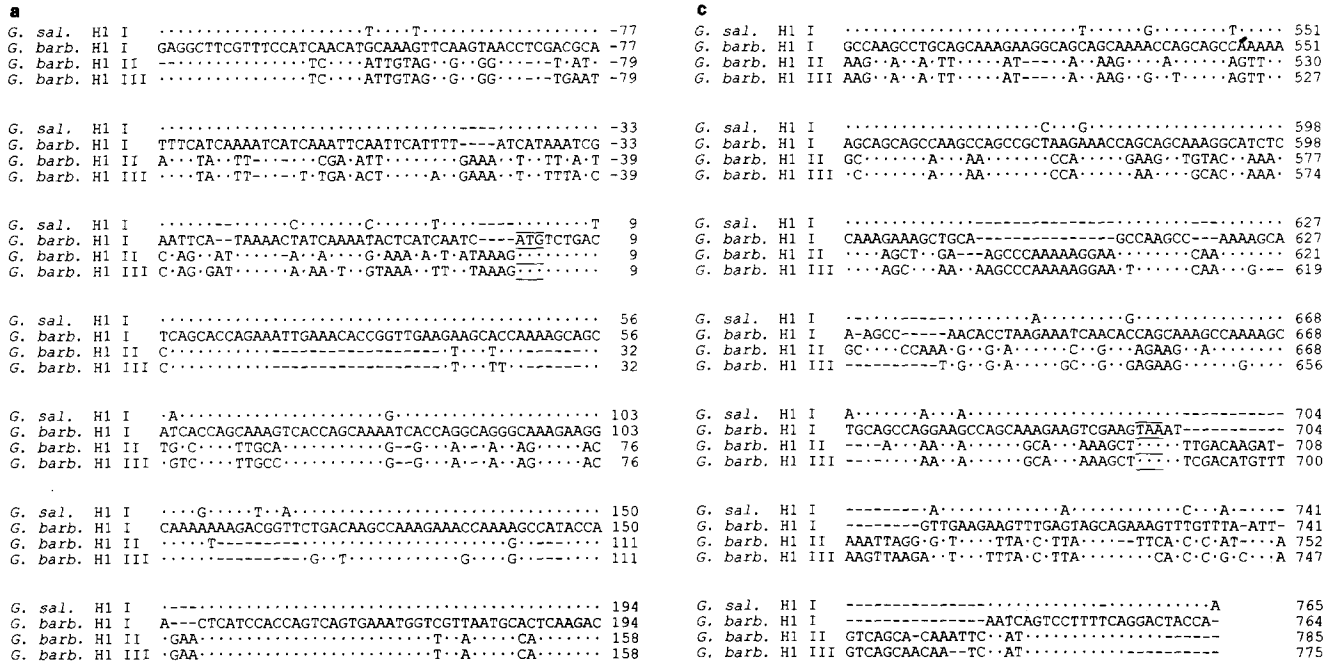


Figure 6. Nucleotide sequences of the histone H1 I, H1 II, and H1 III genes of *G. barbipes* and the histone H1 I gene of *G. salinus*. Dots indicate sequence identities. Start and stop codons are underlined. These sequence data are available from EMBL/GenBank under the accession numbers L29101, L29102, L29103, and L29104, respectively.

H1 I-1 (B. Schulze, 1992; E. Schulze, 1992; Trieschmann, 1992; for details see Materials and Methods). In contrast, the mere hairpin-loop sequence common to H1 genes was used to prime the amplification of all types of H1 genes in the genome. A conserved 5′ sequence common to all known *Chironomus* H1 genes near position −120 (see Materials and Methods) was used as a primer in both types of H1 gene amplification. The amplified DNA was isolated from agarose gels after electrophoresis, cloned in pUC 18, and sequenced using both reverse and inverse pUC 18 primers and an oligonucleotide hybridizing to an evolutionarily conserved sequence coding for a section of the central domain of H1 (E. Schulze, 1992).

Three clones containing different *G. barbipes* H1 genes were sequenced. One of them was the PCR product obtained by priming with the 3′ sequence unique to the I-1 subtype of *Chironomus* H1, and was designated *G. barbipes* H1 I. The two others (H1 II and H1 III) were obtained by PCR with primers suited for amplification of all types of *Chironomus* H1 genes. A *G. salinus* H1 gene amplified using the H1 I-1 specific primer was also sequenced and was designated *G. salinus* H1 I.

The alignment of the four H1 genes (Fig. 6) and their deduced amino acid sequence (Fig. 7) shows that they belong to two types that exhibit remarkable sequence differences. While the H1 II and H1 III genes of *G. barbipes* are rather similar both in the coding and flanking regions, they differ conspicuously from H1 I. On the protein sequence level, ~30% of the deduced amino acid residues are unique to H1 I. Substitutions of amino acid residues in H1 I versus H1 II and H1 III are especially frequent within the NH_2-terminal domain but are not rare even in the central domain that is the most conserved part of H1. The 5′ region between the conserved box used for PCR priming and the start codon exhibits a similarly high degree of base substitutions in H1 I versus H1 II and H1 III, and there are two stretches with a high divergency also downstream the stop codon.

Most interesting in regard to the structural properties of H1 types in *Chironomus*, *Caenorhabditis*, and *Volvox* is, however, an insertion in the H1 I gene that encodes the amino acid sequence, SPAKSPGR, within the NH_2-terminal domain, and that is lacking in the other two H1 genes. At its position, this insertion gives rise to the sequence repeat

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G. sal.  H1 I    ··················T······························ 49
G. barb. H1 I    SDSAPEIETPVEEAPKAASPAKSPAKSPGRAKKAKKDGSDKPKKPKAIP 49
G. barb. H1 II   ··P···V-A·A---·V---------------··E··PK·········PR 37
G. barb. H1 III  ··P···V-ASA--V·V---------------··E··PKT·····A··PR 37

G. sal.  H1 I    ················································· 98
G. barb. H1 I    THPPVSEMVVNALKTLNEKGGSSVIAIKKFLVATYKVEIEKLLPFIKKF 98
G. barb. H1 II   ············I···K·R····LQ········Q···DV···A·····Y 86
G. barb. H1 III  ············I···K·R····LQ······A·Q···DVD··A·····Y 86

G. sal.  H1 I    ···-····················· ········K··A······PRP·- 144
G. barb. H1 I    LKG-AVLKGEVLQVKGTGASGSFKM PPPAKKVDRPESAPKKKATKTK- 144
G. barb. H1 II   ···G··V···L··T··K·······L ·AA···-EKVAKK·M-···GE·K 132
G. barb. H1 III  ·R·-··T···L··T··K·······L ·AA···-EKVAKT·-···AGE·K 131

G. sal.  H1 I    REI---·K·--···-···············-·····----··V······ 182
G. barb. H1 I    TRV---ERK--EKK-VVAKKPKPAVEKKAA-KPAAK----KAAAKPAAK 182
G. barb. H1 II   PKAAAKPK·AG···KSI·····A·TAT·V-K··V··STKKQ··V····· 180
G. barb. H1 III  PQAAAKPK·AL···KSI·····A·TAT·V-K··V··STKKP··V····· 179

G. sal.  H1 I    ······-···E·····-·-·········-·-------········A··· 220
G. barb. H1 I    KAAAKP-AAKKPAAKA-S-PKKAAAKPK-A-------KPTPKKSTPAKP 220
G. barb. H1 II   ···P··K·V--·KP··A-K···E----·K·AAPKAAK··AQ·------- 220
G. barb. H1 III  ···P··K·---·KP··ATK···EV-···KVAA----K··AE·------- 216

G. sal.  H1 I    ····K···-···· 232
G. barb. H1 I    KAAARKPA-KKSK 232
G. barb. H1 II   ··T-K···A··A- 231
G. barb. H1 III  E··-K···A··A- 231
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Figure 7. Deduced amino acid sequences of the H1 histones H1 I, H1 II, and H1 III of *G. barbipes* and H1 I of *G. salinus*. The central globular domains are boxed, and the repeats of the SPAK sequence are underlined. Dots indicate amino acid residues identical with those in H1 I of *G. barbipes*.

SPAKSPAKSPGR. Within H1 I of *G. barbipes*, this repeat lies at almost the same distance from conserved amino acid residues in the central domain as does the KAP repeat in H1 I-1 of *Chironomus* (Schulze et al., 1993) and in the divergent H1 variants of *Caenorhabditis* (Vanfleteren et al., 1988, 1990) and *Volvox* (Lindauer et al., 1993). The H1 I gene of *G. salinus* (Figs. 6 and 7) is very similar to that of *G. barbipes*, with the exception of a sequence encoding a stretch of 20 amino acid residues in the COOH-terminal domain. *G. salinus* H1 I also contains the SPAK repeat. Interestingly, it comprises the only amino acid residue substitution versus *G. barbipes* H1 I outside the COOH-terminal domain and reads TPAKSPAKSPGR (Fig. 7). A data bank search did not yield any known protein sequence that contains a SPAKSPAK repeat.

It is worthwhile to note that a cell type with highly condensed chromatin, the sea urchin sperm cell, contains a specific type of H1 that comprises a structurally similar motif, the SPKKSPKK repeat, at a homologous position within the NH_2-terminal domain (Suzuki, 1989; Wells et al., 1989). A peptide of sea urchin sperm H1 containing repeats of SPKK has been reported to compete with the drug Hoechst 33258 for DNA binding (Suzuki, 1989). The *Glyptotendipes* H1 motif [ST]-PAK SPAK SPGR might be a new

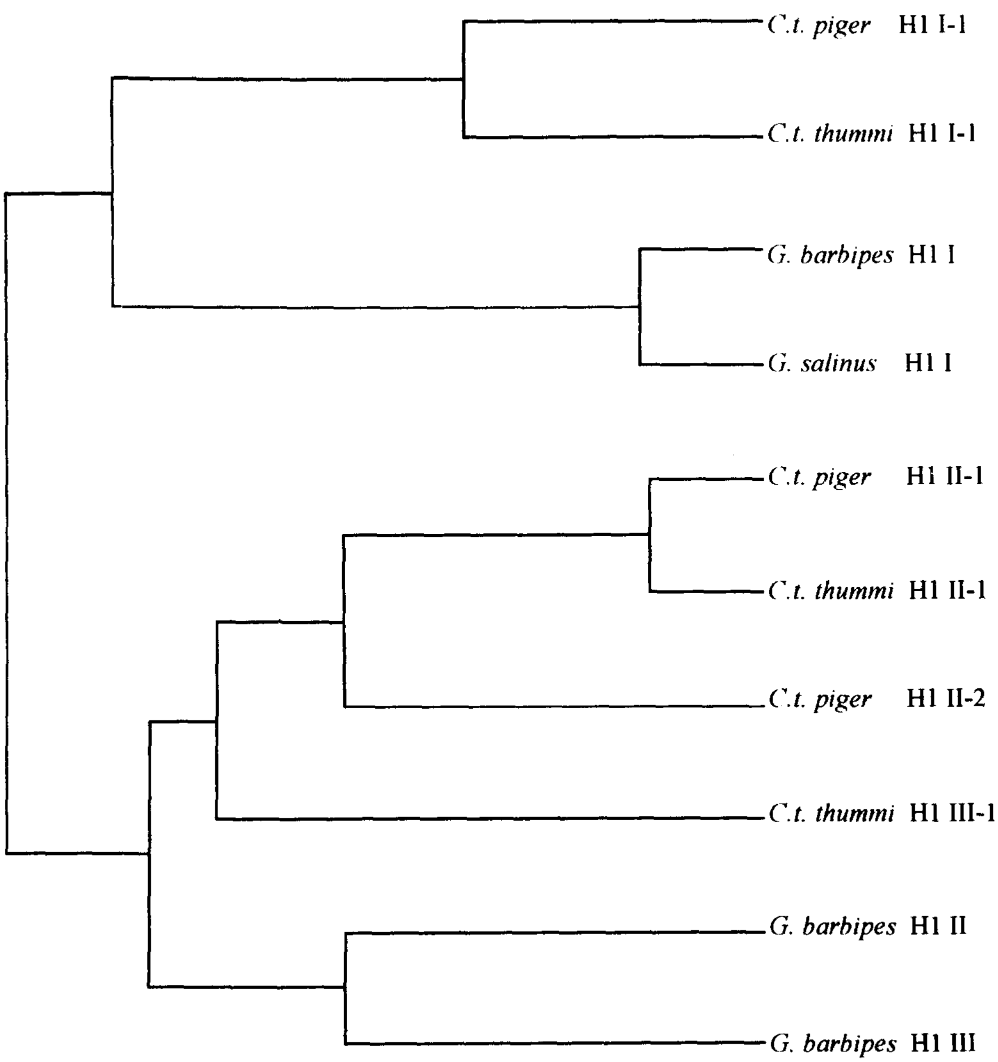

Figure 8. Dendrogram based on the similarities of the *Glyptotendipes* H1 histones to each other and to the H1 histones of *Chironomus thummi* shows the divergency of the two groups of H1 histones within one species and within the two genera. The graph was constructed by the CLUSTAL algorithm (Higgins and Sharp, 1988) which calculates the average similarities of pairs of sequences. For the accession numbers of the *Glyptotendipes* sequences see Legend of Fig. 6. The sequence data of the *C. thummi thummi* and *C. thummi piger* H1 genes are from Schulze et al. (1993) and from our unpublished results and are available from EMBL/GenBank under the accession numbers L28724 to L28732.

member of the group of proline rich basic peptides supposed to interact with a particular DNA structure (Churchill and Travers, 1991).

The occurrence of two structurally divergent groups of H1 proteins is not restricted to the genus *Glyptotendipes*. In *Chironomus thummi*, a remotely related member of the Chironomid family, a similar structural divergency is observed between histone H1 I-1 on the one hand and the other three larval H1 proteins on the other hand (Schulze et al., 1993, and unpublished results). In *Chironomus thummi*, the H1 I-1 gene, in contrast to the H1 genes encoding the other H1 variants, is a single-copy gene located in a different chromosome (Schulze et al., 1993). When the sequences of the H1 proteins of the two organisms are compared, an overall similarity of the sequences of H1 I in *G. barbipes* and *G. salinus* with H1 I-1 of *C. th. thummi* and *C. th. piger* is found that places these proteins much closer to each other than to any member of the other group of intraspecific H1 proteins. A dendrogram (Fig. 8) constructed by the CLUSTAL algorithm (Higgins and Sharp, 1988) as implemented by the program PC/Gene (IntelliGenetics, Inc., Geel, Belgium) on the basis of pairwise similarities between sequences showed that the proteins of the H1 I group in both genera are more similar to each other than to the other H1 proteins. The individual H1 proteins of the H1 II and H1 III type, on the other hand, are most similar to the other proteins of this group in both genera. The presence of two structurally divergent groups of H1 proteins can be considered a homologous property of both genera.

Concluding Remarks

In *Glyptotendipes*, the occurence of conspicuous regions of condensed interphase chromatin on the one hand and the presence of a structurally divergent H1 subtype on the other is at present a mere correlation. The inaccessibility to antibodies of the histones in the centromere and other regions makes it difficult to check in situ whether H1 I is specifically associated with these structures, such as was shown by specific antibodies to be the case for H1 I-1 in condensed chromosome bands of *Chironomus thummi* (Schulze et al., 1993). We have therefore chosen to produce H1 peptides that contain the SPAK repeat and can be used for experiments on interactions with DNA, nucleosomes, and other nuclear proteins.

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