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. barbipes* 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 SPAKSPAKSPGR. 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 SPKKSPPK 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. th. piger*, a difference also evident from a number of homologous chromosome bands in both subspecies that are immunodecorated by HI 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 HI 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 NH₂-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...
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 NH₂-terminal domain creates the protein motif, SPAK-SPAKSPGR, in G. barbipes and TPAKSPAKSPGR in G. salinus. Both structures are similar to those of the KAPPKF 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 condensated 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 oesin-carmine 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 for 3 min in 10% formaldehyde and 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. The DNA was stained with a 4,6-diamidino-2-phenylindole dye Hoechst 33342, washed repeatedly in Tris-buffered saline, and mounted in Tris-buffered saline-glycerol (4:1) 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. The DNA was stained with a 4,6-diamidino-2-phenylindole dye Hoechst 33342, washed repeatedly in Tris-buffered saline, and mounted in Tris-buffered saline-glycerol (4:1) containing 0.1% p-phenylenediamine. Photomicrographs were made on Kodak Tri X Pan film through a Zeiss fluorescence microscope.

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 Kaech, 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 nonolifer 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 in 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 mA per capillary in 10% 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 Amlon 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 (4:1) containing 0.1% p-phenylenediamine. Photomicrographs were made on Kodak Tri X Pan film through a Zeiss fluorescence microscope.

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 GAAGCTTCGTTTGTCCCAATTTTGTAATCGGAGACT 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 (Schulze, 1992; Schulze, 1992; Trieschmann, 1992). In contrast, oligonucleotide TTGTTAGTCCTGAAAAGGACTQA contains two bases (underlined) specific for the 3' sequence of the structurally divergent H1 I gene of C. thummi and its homologue H1e in C. thumus and should thus selectively prime amplification of H1 genes sharing this sequence 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 (Saikl et al., 1988) was...
performed for 45 cycles (annealing temperature 46°C) in a Trio-Thermoblock (Biometa, 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 meta-centric 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 a 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.](image-url)
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 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-isothiocyanate-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.

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.
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 HI 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

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**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.
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. Steuer-nagel, 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 NH2-terminal, central globular, and COOH-terminal domains of the H1 molecule (A. Steuer-nagel, 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.

**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 Chirono-mus. To amplify specifically H1 genes that share sequence peculiarities of the H1-I 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...
HI I (B. Schulte, 1992; E. Schulte, 1992; Trieschmann, 1992; for details see Materials and Methods). In contrast, the mere hairpin-loop sequence common to HI genes was used to prime the amplification of all types of H1 genes in the genome. A conserved 3' sequence common to all known Chironomus HI genes near position -120 (see Materials and Methods) was used as a primer in both types of HI 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 HI (E. Schulte, 1992).

Three clones containing different G. barbipes HI 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 HI, and was designated G. barbipes HI I. The two others (HI II and HI III) were obtained by PCR with primers suited for amplification of all types of Chironomus HI genes. A G. salinus HI gene amplified using the HI I-1 specific primer was also sequenced and was designated G. salinus HI I.

The alignment of the four HI 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 HI II and HI III genes of G. barbipes are rather similar both in the coding and flanking regions, they differ conspicuously from HI I. On the protein sequence level, ~30% of the deduced amino acid residues are unique to HI I. Substitutions of amino acid residues in HI I versus HI II and HI III are especially frequent within the NH2-terminal domain but are not rare even in the central domain that is the most conserved part of HI. The 5' region between the conserved box used for PCR priming and the start codon exhibits a similarly high degree of base substitutions in HI I versus HI II and HI III, and there are two stretches with a high divergency also downstream the stop codon.

Most interesting in regard to the structural properties of HI types in Chironomus, Caenorhabditis, and Wolvax is, however, an insertion in the HI I gene that encodes the amino acid sequence, SPAGPGR, within the NH2-terminal domain, and that is lacking in the other two HI genes. At its main, and that is lacking in the other two HI genes. At its

Figure 6. Nucleotide sequences of the histone HI I, HI II, and HI III genes of G. barbipes and the histone HI 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.
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.

SPA K S P A K S P A K S P G R. 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-I 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 T P A K S P A K S P G R (Fig. 7). A data bank search did not yield any known protein sequence that contains a SPAK S P A K 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 SPK K S P K K repeat, at a homologous position within the NH2-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
The occurrence of two structurally divergent groups of HI proteins is not restricted to the genus Glycophotodes. In Chironomus thummi, a remotely related member of the Chironomid family, a similar structural diversity is observed between histone HI I-1 on the one hand and the other three larval HI proteins on the other hand (Schulze et al., 1993, and unpublished results). In Chironomus thummi, the HI I-1 gene, in contrast to the HI genes encoding the other HI variants, is a single-copy gene located in a different chromosome (Schulze et al., 1993). When the sequences of the HI proteins of the two organisms are compared, an overall similarity of the sequences of HI I in G. barbipes and G. salivaus with HI 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 HI 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 HI I group in both genera are more similar to each other than to the other HI proteins. The individual HI proteins of the HI II and HI 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 HI proteins can be considered a homologous property of both genera.

Concluding Remarks

In Glycophotodes, the occurrence of conspicuous regions of condensed interphase chromatin on the one hand and the presence of a structurally divergent HI 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 HI I is specifically associated with these structures, such as was shown by specific antibodies to be the case for HI I-1 in condensed chromosome bands of Chironomus thummi (Schulze et al., 1993). We have therefore chosen to produce HI peptides that contain the SPAK repeat and can be used for experiments on interactions with DNA, nucleosomes, and other nuclear proteins.

We thank Dr. P. V. Michailova for providing egg-masses of G. barbipes and G. salivaus and for teaching us how to breed these organisms. We are grateful to Ms. I. Streichhan for expert technical assistance and for preparing the Figs. and to Ms. K. Wedekind for preparing the manuscript. This work was supported by a grant from Deutsche Forschungsgemeinschaft to U. Grossbach (Gr 376/11-1).

Received for publication 7 September 1994 and in revised form 10 October 1994.

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