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Structural and functional differences between histone H1 sequence variants with differential intranuclear distribution

(chromatin structure/DNA–protein binding/protein sequence motifs/interphase chromosomes/*Chironomus*)

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ABSTRACT **The chromatin of most cell types contains several different sequence variants of histone H1. The functional role of this heterogeneity is not known. In the larval tissues of the midge, *Chironomus thummi*, there are H1 variants of two types. H1 II-1, H1 II-2, and H1 III-1 have similar amino acid sequences and appear uniformly distributed in polytene interphase chromosomes. The total number of gene copies per genome for this type of H1 histones is about 40 in *C. th. thummi* and 50–60 in *C. th. piger*. In contrast, histone H1 I-1 is encoded by a single copy gene in *C. th. thummi* and by two to four genes in *C. th. piger*. It has a divergent structure and is found only in a limited number of condensed chromosome sites. The N-terminal domain of H1 I-1 contains an insertion that is lacking in the other H1 variants and that is part of a variant-specific bipartite sequence Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala in front of the conserved central domain. N-terminal peptides of H1 I-1 including this motif, in contrast to the homologous peptide from H1 II-1, competed with the drug Hoechst 33258 for binding to the minor groove of the DNA double helix. Repeats of the sequence Lys-Ala-Pro are also present at the same distance from the conserved central domain, in a single H1 variant of a nematode and of a green alga. The motif could interact with linker DNA in intranuclear targeting or packaging a condensed subtype of chromatin, or both.**

The folding of the nucleosome chain into the 30-nm fiber of chromatin is mediated by the binding of histone H1 (1). *In vitro* studies have shown that H1 is a repressor of transcription and that its association with nucleosomes is lost or altered during the transcriptional process (for reviews, see refs. 2 and 3). On the other hand, *in situ* decoration of giant chromosomes with monoclonal antibodies and subsequent ultrastructural analysis have revealed that the chromatin of highly active Balbiani ring genes contains histone H1 (4). These contradictory results as well as other evidence indicate that the role of H1 in the architecture of chromatin may be less uniform than is usually assumed. Within one nucleus, certain H1 subtypes might repress transcription while others may not interfere with this process. Several different sequence variants of H1 have been found in most eukaryotes that have been analyzed carefully (reviewed in refs. 5–7), and indirect evidence suggests a functional role of this diversity (6). Until now, however, direct experimental evidence for a distinct intranuclear location of different H1 subtypes and for functional differences between them has been lacking.

Using subtype-specific monoclonal antibodies, we have shown (8) that different H1 sequence variants are differentially distributed within the salivary gland nuclei of the midge *Chironomus thummi*. Several of the H1 variants of this species appear to be present throughout the polytene chromosomes, whereas H1 I-1, the variant with the most diverging structure (9), was detected only in a limited number of chromosome bands. Many of these bands replicate late in S-phase (10) and contain repetitive DNA (11, 12). They are more frequent in *C. th. thummi* than in *C. th. piger*. Accordingly, H1 I-1 was found to account for about 20% of total H1 in larvae of *C. th. piger* and for about 30% in larvae of *C. th. thummi* (8).

The demonstration within one nucleus of differences between chromosome sites in their H1 subtype content makes *C. thummi* a model organism for studying possible functions of H1 heterogeneity. Therefore, we have determined and compared the sequences of the different H1 histone genes of *C. thummi* in a search for structural features that are peculiar to H1 I-1 and could be involved in establishing a particular subtype of chromatin. We find that H1 I-1, in contrast to the other H1 variants, contains a novel sequence motif that is not known from other proteins but is also present in single H1 variants of other, evolutionarily distant organisms. An N-terminal peptide of H1 I-1 that includes this motif also differs from the homologous region in another H1 variant of *C. thummi* by competing with the drug Hoechst 33258 for binding to the minor groove of the DNA double helix. Interaction of the subtype-specific motif with linker DNA may play a role in intranuclear targeting of H1 I-1 or in establishing a condensed type of chromatin, or in both.

MATERIALS AND METHODS

Clone Isolation and DNA Sequencing. Genomic clones containing histone gene sequences were identified in a *C. th. thummi* library (13) by a cloned histone gene repeating unit from *Drosophila melanogaster* (14), and *Hin*dIII restriction blots were screened for H1 sequences by an oligonucleotide (15-mer with 32 sequence variants) deduced from the pentapeptide, Ser-Gly-Ser-Phe-Lys, that represents a sequence highly conserved in H1 proteins. One *Hin*dIII fragment containing most of the coding region of an H1 gene and one *Acc* I fragment containing the entire gene with its 5′ and 3′ flanking regions were subcloned in the pUC18 vector and sequenced. The *Hin*dIII fragment was also used as a probe for screening a genomic *C. th. piger* library constructed by L.T.; the DNA of 16 positive clones was restricted by *Hin*dIII, separated on agarose gel, transferred to Hybond-N (Amersham) according to the instructions of the manufacturer, and hybridized at high stringency with a pUC18 subclone of the *Hin*dIII fragment that contains 400 base pairs (bp) of the H1 gene coding for parts of the central and N-terminal domains. Appropriate restriction fragments con-

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taining H1 genes were subcloned in the pUC18 vector and sequenced. All sequencing was performed by the dideoxy method (15).

A gene probe specific for H1 I-1 of *C. th. piger* (see *Results*) was used to screen a genomic *C. th. thummi* library made by B.S., and a positive clone containing the *C. th. thummi* H1 I-1 gene was subcloned and sequenced.

Isolation and Purification of H1 Peptides. Histone H1 variants I-1 and II-1 from larvae of *C. th. thummi* were isolated as described (9), purified by reversed-phase HPLC, and cleaved with BrCN at their single methionine residue. In both proteins, the cleavage yielded a short N-terminal and a long C-terminal peptide. The N-terminal peptide of each protein was purified by reversed-phase HPLC and used in the DNA binding experiments. The N-terminal peptide of H1 variant I-1 from *C. th. piger* was obtained by expression in an *E. coli* expression system that uses bacteriophage T7 RNA polymerase (16).

DNA Binding in Competition with Hoechst 33258. Salmon sperm DNA (82.3 nM in phosphate residues) was preincubated with Hoechst 33258 (Hoechst) at a concentration of 10.9 nM. The fluorescence intensity of the solution was measured at 25°C in 10 mM sodium phosphate buffer, pH 7.1/150 mM KCl in a Perkin–Elmer LS 50 spectrofluorimeter at 450 nm (excited at 354 nm). The true fluorescence, F, of the DNA–dye complex was calculated by subtracting the background fluorescence of all components minus dye, and the fluorescence of the dye and all components minus DNA. Increasing amounts of peptide were then added, and the decrease of F was expressed in percent relative to the true fluorescence of the DNA–dye complex without peptide.

Estimation of Gene Copy Numbers. Histone H1 gene probes were hybridized on Southern blots to equivalent molar amounts of genomic DNA and plasmid DNA. For calibration, a series of plasmid DNA concentrations equivalent to increasing numbers of gene copies per genome was used, based on genome sizes of 2.8×10^8 bp (*C. th. thummi*) and 2.18×10^8 bp (*C. th. piger*) as determined by H. G. Keyl (personal communication). DNA of plasmid clones containing the complete coding sequence of H1 genes I-1, II-1, and II-2, respectively, and different stretches of flanking sequences were digested with restriction enzymes to yield the genomic insert and were separated on agarose gel together with genomic DNA. Southern blotting onto Hybond-N was performed as described above. Gene probes were as follows: probe 1, a 319-bp *Pst* I–*Bam*HI fragment encoding the C-terminal half of H1 I-1 of *C. th. piger* [specific for H1 genes of type I-1 in both subspecies (L.T. and B.S., unpublished data)]; probe 2, a 4-kbp *Hin*dIII fragment encoding the entire H1 I-1 gene of *C. th. thummi* with flanking sequences; probe 3, a 400-bp *Hph* I fragment encoding the central domain and part of the N-terminal and C-terminal domains of H1 II-1 of *C. th. thummi*. At high stringency, probe 3 hybridized with the H1 II-1, H1 II-2, and H1 III-1 genes but not with H1 I-1 genes (L.T., unpublished data). The probes were labeled with digoxigenin-11-dUTP, and hybridization was visualized by a chemiluminescence reaction with the DIG luminescent detection kit (Boehringer Mannheim). In another series of experiments, the probes were labeled with [^{32}P]dNTPs by the random primer method (17), and hybridization was visualized by autoradiography. Labeled spots corresponding to fragment(s) of genomic DNA and plasmid inserts were cut from the membrane, the radioactivity was counted in a liquid scintillation counter, and the results were used to construct a calibration curve to determine the number of gene copies per genome.

RESULTS AND DISCUSSION

To address the question of functional differences within a nucleus between sequence variants of histone H1, we have earlier characterized the H1 subtypes in larvae of *C. thummi*. Seven H1 fractions obtained by two-dimensional electrophoresis were analyzed by comparing the peptide patterns after chemical and enzymatic cleavage and by isolation of their folded central domains. From these results (9) it appeared that most and probably all fractions were sequence variants of H1. However, HPLC of the H1 histones and their peptides and microsequencing of selected peptides have recently shown that there are altogether four different H1 histones in *C. thummi* larvae (E.S. and J. R. Wisniewski, unpublished results).

This analysis became possible when the H1 genes of *C. thummi* had been sequenced (see below). Peptides that contained a putative amino acid sequence specific for the gene products of the individual H1 genes were isolated and sequenced. The results revealed the identity and the degree of homogeneity of the individual electrophoretic H1 fractions. Three of them (H1 I-1, H1 II-1, and H1 III-1) were found to represent single polypeptide chains, two (II-2 and III-2) were a mixture of a fourth polypeptide (which we denominate II-2) and a degradation product of II-1, while the remaining fractions represented degraded H1 polypeptides (E.S. and J. R. Wisniewski, unpublished results).

The four H1 histones were regularly detected in individual larvae from inbred strains by small-scale two-dimensional electrophoresis (H. Leufgen and U.G., unpublished results). Therefore, their appearance is probably not due to an allelic polymorphism.

According to the number of H1 variants, four types of H1 genes were found in *C. thummi* (see below). As H1 variant I-1 exhibited a divergent protein structure (9) and was detected only in a minority of chromosome bands (8), we were especially interested in structural properties that are peculiar to H1 I-1 and that may be involved in specific functions.

Histone H1 I-1 Contains an Inserted Sequence Motif. We have compared the primary structure of H1 I-1 to the structures of the other H1 variants. For this purpose, the sequences of genes encoding the H1 histones I-1 and II-1 of both subspecies and II-2 and III-1 of *C. th. piger* were determined. Clones encoding single amino acid substitutions of variant II-1 were also sequenced in part. The individual H1 genes were identified by comparing their deduced amino acid sequences to sequences of suitable peptides of the H1 proteins. The deduced amino acid sequences of the N-terminal domains and part of the central domains have been aligned in Fig. 1 and show a conspicuous feature of histone H1 variant I-1. In both subspecies, the N-terminal domain of this protein differs from the homologous domains of the other H1 histones by a sequence that consists of two parts: (*i*) the insertion of a repeat of the sequence Lys-Ala-Pro, which is reiterated five times (three direct and two modified repeats) in the *C. th. piger* and four times (two direct and two modified repeats) in the *C. th. thummi* protein; and (*ii*) the sequence Lys-Val-Ala that lies 10 amino acid residues apart from the Lys-Ala-Pro repeat and replaces a single Lys-Ala-Pro that is characteristic of the other H1 proteins of *C. thummi* at the same position (Fig. 1). An additional H1 gene of *C. th. thummi* (13), which we have identified as H1 III-1, also lacks the Lys-Ala-Pro repeat and contains a single Lys-Ala-Pro at the Lys-Val-Ala site (13). The bipartite Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif is thus specific for variant H1 I-1 and is not present in the other H1 proteins of *C. thummi*.

While the sequence Lys-Ala-Pro is rare in histones and a data bank search has not revealed other known proteins containing the sequence Lys-Ala-Pro-Lys-Ala-Pro, we have identified the bipartite Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif in one of the three published H1 histone sequences of the nematode *Caenorhabditis elegans* (18–20). Here the motif lies at exactly the same distance as in *Chironomus* H1 from the conserved sequences in the central

H1	clone		↓
I-1(p)	L1234	SDPAPEIEAPVEAAPVASPPKGKKEK	APKAPKAPKSPKAEKPKSDKPKKPKVAPTHPPVSEMVVNAVTTL
I-1(t)	T31	SDPAPEIEAPVEAAPVASPPKGKKE	KAPKAPKSPKAEKPKSDKPKKPKVAPTHPPVSEMVVNAVTTL
II-1(t)	TL4	SDPAVEV APTT PVASPAKAKKEK	KPKTDKVKKPKAPRTHPPVSEMVVNAVKTL
II-1(p)	L145	SDPAVEV APTT PVASPAKAKKEK	KPKTDKPKKPKAPRTHPPVSEMVVNAVKTL
II-1(p)	L91	SDPAVEV ARTT PVASPAKAKKEK	KPKTDKPKKPKAPRTHPPVSEMVVNAVKTL
II-1(p)	L92	.....V APTT PVASPAKAKKEK	KPKTDKVKKPKAPRTHPPVSEMVVNAVKTL
II-1(p)	L95	SDPAVEV APTT PVASPAKAKKEK	KPKTDKPKKPKAPRTHPPVSEMVVNAVKSL
II-2(p)	L1254	SDSAVDV APTT PVASPAKAKKEK	KPKTDKPTKPKAPKTHPPVSEMVVNAVKTL
III-1(p)	L1413	SDPAIEV APV PVASPAKAKKEK	KPKSDKPKKPKAPRTHPPVSDMIVNAIKTL

FIG. 1. The histone H1 variants of *C. thummi* larvae. The derived amino acid sequences of the N-terminal domains and adjacent portions of the globular domains are shown in alignment. Variants of the two subspecies *C. th. thummi* and *C. th. piger* are marked by (t) and (p), respectively. The inserted Lys-Ala-Pro (KAP) repeats, the sequence KAP, the sequence Lys-Val-Ala (KVA) that is thought to form part of a bipartite motif KAPKAPX$_{10}$KVA, and the methionine residues are underlined. Dots indicate an unknown sequence, and the arrow indicates the border between the N-terminal and globular domains.

domain of the molecule (Fig. 2). Its two parts are also separated by 10 amino acid residues, but these show a very low degree of homology between *Chironomus* and *Caenorhabditis*. Again, the Lys-Val-Ala part of the motif is replaced by Lys-Ala-Pro in the other H1 sequences of this organism (Fig. 2). Interestingly, the Lys-Ala-Pro repeat is also found inserted, at almost the same distance from a Lys-Ala-Ala sequence and from the conserved region in the central domain, in the deduced amino acid sequence of one of the two known H1 genes of the alga *V. carteri* (21). Here it has the sequence Lys-Ala-Pro-Lys-Gln-Pro-Lys-Ala-Pro-Lys-Ala-Pro and is flanked by additional Lys-Ala-Pro elements (Fig. 2). Again, the second H1 gene in *V. carteri* that has been sequenced does not contain a repeat of Lys-Ala-Pro (21). We have thus identified a protein motif that has been conserved in evolution in very distant organisms and that appears in only one of their H1 histones. As the N-terminal domains of H1 histones exhibit a high degree of interspecific variability (22), the presence of the Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif at a specific position in one of the H1 histones of a midge, a nematode, and a green alga is not trivial and may suggest a functional role of this sequence.

Competition of the N-Terminal Domain with Hoechst 33258 for DNA Binding. In *Chironomus* the H1 variant with the Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif is targeted to a minority of interphase chromosome bands. Many of the bands that contain the H1 variant I-1 have properties of highly condensed chromatin (8). A possible function of the motif thus could be to bind variant I-1 to specific chromatin sites or to contribute to packaging a condensed subtype of chromatin, or both. We have asked whether the amino terminus of this H1 protein interacts with DNA in a way that is specific for its structure and therefore have compared the binding to DNA of the N-terminal peptides of H1 I-1 and H1 II-1 in competition with Hoechst 33258. This drug binds in the minor groove of the DNA double helix in a selective and well-characterized way (23, 24). It competes for DNA binding with an N-terminal peptide of sea urchin H1 (25). H1 I-1 and H1 II-1 from larvae of *C. th. thummi* were cleaved with BrCN, and their N-terminal peptides (amino acid residues 1–59 and 1–45, respectively; cf. Fig. 1) were used in the experiment. The N-terminal peptide of H1 I-1 from *C. th. piger* (amino acid residues 1–62) was obtained in larger amount by expression in an *E. coli* expression system that uses bacteriophage T7 RNA polymerase (16). The peptides were added to DNA that had been preincubated with Hoechst 33258 in a concentration range where the dye binds specifically to the minor groove (25, 26). Both H1 I-1 peptides that contain the Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif were found to compete with Hoechst 33258 for binding to DNA (Fig. 3). In contrast, the homologous BrCN peptide of histone H1 II-1 that does not contain the bipartite Lys-Ala-Pro motif but otherwise has a very similar sequence (cf. Fig. 1) did not compete with Hoechst 33258 for DNA binding under these conditions (Fig. 3). The synthetic peptide Lys-Ala-Pro-Lys-Ala-Pro-Lys-Ala-Pro-Lys-Ser-Pro-Lys-Ala also showed little competition with Hoechst 33258 (not shown). We presume that the N-terminal domain of H1 I-1 binds to the minor groove of the DNA double helix *in vivo*, whereas the homologous domains of the other H1 variants do not, and that this binding is mediated by the Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif. The Lys-Ala-Pro-Lys-Ala-Pro peptide, on the other hand, may not be sufficient but may function in a specific neighborhood that may require the Lys-Val-Ala sequence at a defined distance and conserved structure(s) in the central domain of the H1 molecule. The Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala motif in the N-terminal domain is located in immediate neighborhood to the central globular domain of the H1 molecule (Fig. 2). As the globular domain binds to the nucleosome (27), the motif may interact with a stretch of linker DNA close to where it enters the histone octamer. This interaction could help to establish a condensed subtype of chromatin. We are in the course of investigating the site and mode of interaction with DNA *in vitro* and by microinjection into polytene nuclei.

FIG. 2. Alignment of the KAPKAPX$_{10}$KVA motifs in single H1 variants of *C. thummi* (row A1), *Volvox carteri* (row B3), and *C. elegans* (row C5). The structures common to the other H1 variants of *C. thummi* (row A2) and *Caenorhabditis* (row C6) and the structure of the other known H1 variant of *Volvox* (row B4) are shown for comparison. The adjacent portions of the globular domains are boxed. Numbering refers to the amino acid residues between the N-terminal serine and alanine, respectively, the KAPKAPX$_{10}$KVA motif, and conserved residues in the globular domain. The *Caenorhabditis* data is taken from refs. 18–20, and the *Volvox* data, from ref. 21.

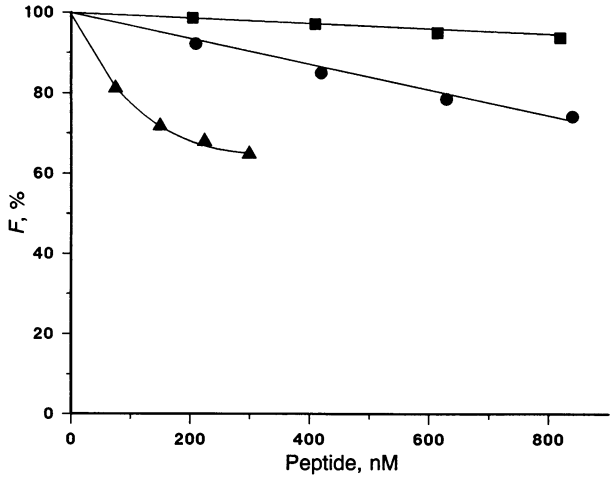

FIG. 3. Competition of H1 peptides containing the Lys-Ala-Pro-Lys-Ala-Pro-Xaa$_{10}$-Lys-Val-Ala (KAPKAPX$_{10}$KVA) motif with Hoechst 33258 for DNA binding. Increasing amounts of N-terminal peptides of H1 I-1 (residues 1–59) from *C. th. thummi* larvae (▲), bacterially expressed H1 I-1 (residues 1–62) from *C. th. piger* (●), and H1 II-1 (residues 1–45) from *C. th. thummi* (■) were added to DNA preincubated with the drug. The true fluorescence, *F*, of the DNA–dye complex was calculated from the measured fluorescence. Its decrease after the addition of peptide is expressed in percent of *F* of the complex without peptide.

An N-terminal peptide of H1 from sea urchin sperm that is 48 amino acid residues long and contains repeats of the protein motif Ser-Pro-Lys-Lys, has been reported to compete with Hoechst 33258 for DNA binding (25). It is interesting to note that the direct Ser-Pro-Lys-Lys repeats in the sperm H1 histones from three different sea urchin species (22, 25) lie at exactly the same distance from the conserved methionine residue as do the Lys-Ala-Pro repeats in *Chironomus*, *Caenorhabditis*, and *Volvox*. Therefore, it is tempting to speculate that both types of repeats are part of a structure that brings about a condensed subtype of chromatin, such as those typical for total sperm chromatin and for those chromosome sites in *Chironomus* where H1 I-1 is located.

Numbers of Genomic Copies of the Different H1 Genes. Probes specific for H1 I-1 genes and for the group of the other H1 genes, respectively, were used to estimate the numbers of copies present per genome. Surprisingly, the numbers were found to be dramatically different. The results for *C. th. thummi* are shown in Fig. 4 *A* and *B*. The H1 I-1-specific probe 2 hybridized to the H1 I-1 gene in plasmid and genomic DNA (Fig. 4*A*, lanes 1–4) but not to other H1 genes (Fig. 4*A*, lanes 4–7). The hybridization signal of genomic DNA (lane 4) appeared about half as strong as that of a plasmid insert equivalent to two gene copies per genome (lane 3). On the other hand, probe 3 that is specific for the other H1 genes hybridized to the H1 II-1 gene in genomic and plasmid DNA (Fig. 4*B*, lanes 4–8) but not to H1 I-1 (Fig. 4*B*, lanes 1–3). The hybridization signal yielded by genomic DNA (lane 4) appeared to be similar to that obtained with an equivalent to 40 gene copies (lane 7). We conclude that the genome of *C. th. thummi* probably contains one copy of the H1 I-1 gene and about 40 copies of the other H1 genes.

With genomic DNA of *C. th. piger*, probe 1 that is specific for the H1 I-1 gene hybridized to two fragments of 2.5 and 1.0 kbp (Fig. 4*C*, lanes 1 and 2). In both of them, the hybridization signals appeared to be similar to those equivalent to one and two gene copies (Fig. 4*C*, lanes 3 and 4). A quantitative determination of the amounts of probes hybridized to the blots was made by identifying the spots on autoradiograms, cutting them from the membrane, and counting the radioactivity hybridized to genomic and to standard amounts of plasmid inserts in a scintillation counter. The 2.5-kbp fragments from lanes 3–7 of the autoradiogram shown in Fig. 4*C* yielded a linear calibration curve (not shown) for calculation of the amounts of H1 I-1 gene copies in genomic DNA (Fig. 4*C*, lanes 1 and 2). Linear calibration curves (not shown) were also obtained in three independent experiments with gene probe 3 at low stringency in which the total number of

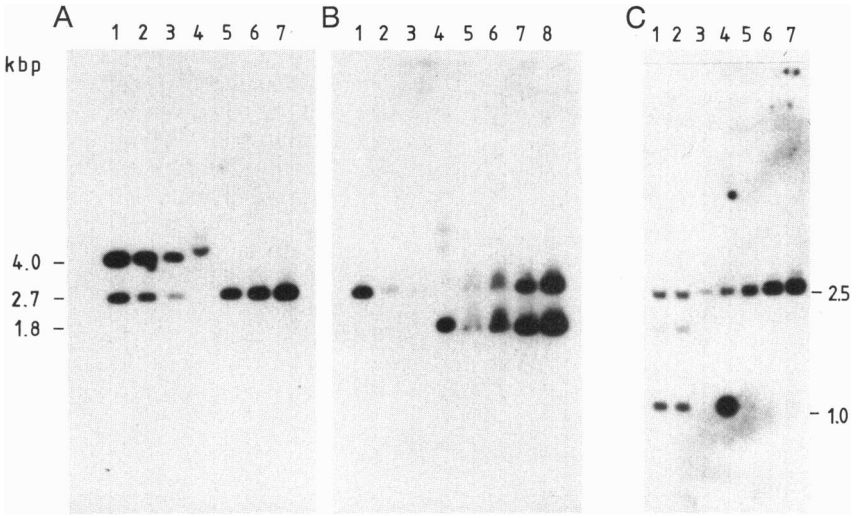

FIG. 4. Copy numbers per genome of the two types of H1 genes. Genomic DNA of *C. th. thummi* (*A* and *B*, lanes 4) and *C. th. piger* (*C*, lanes 1 and 2) was cleaved with restriction enzymes *Acc* I and *Hin*dIII, respectively, and separated on agarose gel in parallel with digested plasmid DNA containing a complete H1 I-1 gene (*A* and *B*, lanes 1–3; *C*, lanes 3–7) or a complete H1 II-1 gene (*A* and *B*, lanes 5–8). The amounts of plasmid DNA were chosen to be equivalent to increasing numbers of gene copies per genome. The gels were blotted onto Hybond-N and hybridized with gene probes 1 and 2 for H1 I-1 (*A* and *C*) or with gene probe 3 that is specific for the other H1 variants (*B*). (*A*) Probe 2 hybridized to a 4-kbp genomic fragment (lane 4) and to a plasmid H1 I-1 insert of corresponding size (lanes 1–3). (*B*) Probe 3 hybridized to a 1.8-kbp genomic fragment (lane 4) and to a plasmid H1 II-1 insert of corresponding size (lanes 5–8). (*C*) Probe 1 hybridized to two genomic DNA fragments of 2.5 and 1.0 kbp (lanes 1 and 2) and to a 2.5-kbp plasmid H1 I-1 insert (lanes 3–7). Five micrograms of genomic DNA was applied in *A* and *B*, and 2.5 μg, in *C*. In *A* and *B*, the plasmid DNA amounts were equivalent to 10, 4, and 2 gene copies per genome in lanes 1–3, respectively, and to 10, 20, 40, and 80 gene copies in lanes 5–8. In *C*, the plasmid DNA amounts in lanes 3–7 corresponded to 1, 2, 5, 10, and 15 gene copies, respectively.

Table 1. Numbers of H1 gene copies per genome in *C. th. piger*

All H1 types*			H1 I-1†		
blot	cpm	genes	lane	cpm	genes
1	536	50	1	45	0.9
2	3272	58	2	50	1.7
3	3292	61			

Radioactivity of the gene probe hybridized to genomic DNA fragment of appropriate size was measured. Gene copy numbers were determined by calibration curves derived from standards on the same blots.
*Independently labeled probes.
†Samples from one blot.

H1 genes was determined, with plasmid DNA containing a copy of the H1 II-2 gene as a standard. The results are shown in Table 1. The genome of *C. th. piger* thus contains about 50–60 copies of the one group of H1 genes and very few copies of the H1 I-1 gene. The numbers obtained from 2.5-kbp fragments of genomic DNA, 0.9 and 1.7 (Table 1), must probably be doubled because another 1.0-kbp fragment of genomic DNA showed hybridization with the H1 I-1 probe of corresponding intensity (Fig. 4*C*, lanes 1 and 2). The genomic fragments of 2.5 and 1.0 kbp may both contain an H1 I-1 gene with a specific genomic organization. Therefore, we assume that *C. th. piger* contains two to four copies of the H1 I-1 gene. *In situ* hybridization has revealed that in both subspecies of *C. thummi*, the H1 genes of the I-1 type are located in another chromosome than are the other 40–60 H1 gene copies in the genome (L.T. and B.S., unpublished results).

Histone H1 variants containing the Lys-Ala-Pro-Lys-Ala-Pro-Xaa_{10}-Lys-Val-Ala motif may be present also in other organisms. The fact that *C. th. thummi* and *C. th. piger* have one copy and a few copies, respectively, of the H1 I-1 gene indicates the necessity to search for rare copies of variant H1 genes. Preliminary experiments with DNA from a number of plant and animal species in which oligonucleotides coding for the Lys-Ala-Pro repeat were used as probes have not been successful. It should be possible, however, to elicit antibodies against the motif and to use them to detect H1 histones of the type found in *Chironomus, Caenorhabditis*, and *Volvox*.

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