Different Behavior of Chromatin Domains Encompassing Fibroin Heavy-chain Gene in Active, Temporarily Inactive, and Permanently Inactive Transcriptional States in Silk Gland Nuclei*

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Chromatin structures of an ~46-kilobase pair region encompassing fibroin heavy chain gene of the silk-worm, Bombyx mori, were compared in active (in the posterior silk gland nuclei of the fifth instar larvae), temporarily inactive (in the posterior silk gland nuclei of the fourth molting stage larvae), and permanently inactive (in the middle silk gland nuclei of the fifth instar larvae) transcriptional states. Chromatin structure of the second exon, the major body of the protein-encoding region, was highly sensitive to both DNase I and methidiumpropyl EDTA-Fe(II) in the active state but highly resistant to those agents in both temporally and permanently inactive states, except that the fibroin "amorphous" region-encoding subregions remained relatively sensitive to DNase I. Distributions of DNase I hypersensitive sites in the 5' upstream region were generally similar between active and temporarily inactive states, but those of the permanently inactive state were markedly different. In the promoter-enhancer region, phosphodiester bonds between nucleotide positions -41 and -42 and also -42 and -43 were remarkably hypersensitive to DNase I in the active chromatin, but the same positions in the other two states were not.

Silk fibroin consists of a heavy (H)1 and a light (L) chain polypeptides of approximately 350 and 25 kDa, respectively, which are linked by a disulfide bond (1, 2). Genes for the H-chain (fibH) and the L-chain (fibL) are located on different chromosomes, but their expression seems to be coordinately regulated in posterior silk gland (PSG)-specific and larval molting cycle-dependent manners (3). Mechanisms of this coordinate expression have not been elucidated, although many common sequence elements were found in an ~1-kb region upstream of transcription start points of fibH and fibL (4). It has been shown that the chromatin structure of the protein encoding region of fibH in the PSG nuclei is unique in that its sensitivity to micrococcal nuclease or DNase I changes periodically through a larval molting cycle, i.e., it is highly sensitive to these nucleases when the gene is actively transcribed during an instar (feeding stage) but becomes highly resistant to these nucleases when the transcription is inactive during a molting stage (5).

In this study, we aimed to compare chromatin structures of a genomic region encompassing fibH in three different transcriptional states, i.e., an active state in the PSG nuclei during the fifth instar, a temporarily inactive state in the PSG nuclei during the fourth molting stage, and a permanently inactive state in the middle silk gland (MSG) nuclei. An ~46-kb region from -27 to +19 kb encompassing fibH was analyzed for sensitivity to DNase I or methidiumpropyl EDTA (MPE)-Fe(II). An enhancer-promoter region was analyzed by in vivo DNA footprinting at the level of a single base pair. These studies revealed that chromatin domains for the far-upstream, enhancer-promoter, and protein-encoding regions behaved differently under different transcriptional states of fibH.

**EXPERIMENTAL PROCEDURES**

Preparation of Silk Gland Nuclei and DNase I Digestion—The ramified nuclei of PSG or MSG were isolated from larvae of Bombyx mori J-139 (a Japanese breed) at a stage indicated as described previously (5), except that crude nuclei were centrifuged twice through a step gradient consisting of 40 and 80% Percoll (Pharmacia). Nuclei recovered from the interface between the two Percoll layers were washed in a digestion buffer consisting of 10 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 0.5 mM CaCl₂, 25 mM KCl, 0.5 mM dithiothreitol, 0.35 M sucrose by resuspension and centrifugation at 4°C for 15 min. DNA content of the nuclei was determined fluorometrically with a DNA-binding dye, Hoechst 33258 (5). Nuclei were resuspended in the digestion buffer at a concentration of approximately 250 or 500 μg DNA/ml and incubated with various concentrations of DNase I for 5 min at 30°C. Reactions were terminated and DNA purified as described previously (5).

Treatment of Nuclei with Methidiumpropyl EDTA-Fe(II) Complex—To prepare an aqueous MPE-Fe(II) reagent, equal volumes of 5 mM MPE and 5 mM ferrous ammonium sulfate were mixed, and the solution was diluted immediately with 10 mM Tris-HCl (pH 7.5), 25 mM KCl, 0.35 M sucrose containing dithiothreitol to give final concentrations of 0.04 mM MPE-Fe(II) and 4 mM dithiothreitol. The suspension of nuclei in the digestion buffer was adjusted to approximately 300 μg of DNA/ml, 10 mM EDTA, and 2 mM hydrogen peroxide, which was then mixed with an equal volume of the aqueous MPE-Fe(II) reagent and the mixture was incubated at 22°C for 10–45 min. Reactions were terminated by adding bathophenanthroline to 5 mM, and DNA was purified as above.

Probes for Hybridization—Probes used in this study are illustrated in Fig. 1. PE1 is the insert of a recombinant plasmid pJ9HPE1 (5). The abbreviated names of these plasmids are given in Table I. KH05 and P2 are inserts of pUC119 recombinant plasmids, corresponding to the EcoRI-HindIII (~5.9 to ~5.4 kb) and the PstI-PstI (~5.4 to ~6.1 kb) sequences, respectively. KH05, P2, and PE1 probes were labeled by the random priming method (6) with [α-32P]dCTP.

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1 The abbreviations used are: H, heavy; L, light; PSG, posterior silk gland; MSG, middle silk gland; kb, kilobase(s); bp, base pair(s); MPE, methidiumpropyl EDTA; PCR, polymerase chain reaction; CR, crystalline; AM, amorphous.
CR is a mixture of three single-stranded 20-mer oligodeoxynucleotides synthesized by a DNA synthesizer (381A, Applied Biosystems): CR1 (upper strand) 5'GGTGAGCTGTTGCTGG-3', CR2 (lower strand) 3'TGCTGGAGACTGCGG-5', and CR3 (upper strand) 5'GATATGGCTGAGATTCG-3'. These sequences correspond to the published fibroin "crystalline" region-encoding sequences (7). AM is a mixture of three single-stranded 20-mer oligodeoxynucleotides synthesized as above: AM1 (upper strand) 5'-AAATGGGCGATAGCGC-3', AM2 (lower strand) 3'TGCTGGAGACTGCGG-5', and AM3 (upper strand) 5'-TATATGGCTGAGATTCG-3'. These regions were selected from the sequences of two clones, obtained in this study, containing 51- and 54-bp AvalI-HinfI fragments from the "amorphous" region-encoding sequences of fibH (8). CR and AM probes were 5'-end-labeled by T4 polynucleotide kinase with [γ-32P]ATP.

Southern Blot Analysis—DNA purified from the nuclei was digested with restriction enzymes as indicated in figure legends and electrophoresed on a 45-cm-long agarose gel (0.6, 1.0, or 1.5%) in 40 mM Tris-acetate (pH 7.8), 2 mM EDTA, 0.5 μg/ml ethidium bromide. After electrophoresis, the gel was immersed in 0.25 M HCl for 15 min, then the DNA in the gel was denatured in 0.4 M NaOH, 0.6 M NaCl for 30 min and transferred to a nylon membrane (GeneScreen Plus (Du Pont-New England Nuclear) or Hybond N+ (Amersham) in the same alkaline salt solution. The membrane was washed in 0.5 M Tris-HCl (pH 7.4), 1 M NaCl for 15 min and air-dried. Prehybridization was carried out in a hybridization buffer (50 mM Tris-HCl (pH 7.5), 1 M NaCl, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate) at 65 °C for at least 20 min. Hybridization was carried out in the hybridization buffer containing 1·× 106 dpm (0.1-0.4 ng)/ml heat-denatured 32P-labeled PE1 or EH05 probe and 200 μg/ml sheared, denatured salmon sperm DNA at 65 °C for 16 h. The membrane was washed at 65 °C in 2·× SSC (1·× SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.5% SDS, then in 0.1·× SSC, 0.5% SDS (for PEI probe) or in 0.3·× SSC, 0.5% SDS (for EH05 probe). When a synthetic oligodeoxynucleotide probe was employed, prehybridization was carried out in a buffer containing 50 mM Tris-HCl (pH 7.5), 1 M NaCl, 1% SDS, 5·× Denhardt’s solution, 200 μg/ml sheared, denatured salmon sperm DNA at 65 °C for 16 h. Hybridization was carried out in the same buffer containing a 32P-labeled probe (1 pmol/ml) at 66 °C (for CR probe) or 42 °C (for AM probe) for 16 h. The membrane was washed successively in 3·× tetramethylammonium chloride buffer acetic acid). The gel was fixed in 10% methanol, 10% acetic acid, dried on Whatman 3MM paper, Regular intensifying screen at -80 °C for 1-7 days.

RESULTS

Contrasting Sensitivity of Transcriptionally Active and Inactive fibH Chromatin to DNase I or MPE-Fe(II)—DNase I sensitivities of a region containing almost the entire second exon of fibH were compared in different silk gland nuclei. Nuclei were digested with increasing concentrations of DNase I, and DNA purified was digested with PstI and subjected to Southern blot hybridization with PEI probe, so that an ~18- kb region from ~0.4 kb downstream from the 5'-end of the second exon to ~3 kb downstream from the 3'-end of fibH, could be examined (Fig. 1). As shown in Fig. 2, this region was susceptible to digestion with less than 0.1 units/ml DNase I when the gene was actively transcribed in PSG nuclei of the fifth instar larvae (lanes 7 and 8), whereas more than 1 unit/ml DNase I was needed to obtain similar extent of digestion when the transcription was temporarily inactive in PSG nuclei of the fourth molting-stage larvae (lane 13) or permanently inactive in MSG nuclei (lane 10). A broad band of about 15.5 kb, which was detectable more clearly in the transcriptionally inactive states (lanes 10 and 14), was interpreted to be produced from the previously identified hyper-sensitive site (HS-1) located just beyond the 3'-end of the gene, i.e. between EcoRI (+16.4 kb) and HindIII (+17.5 kb) sites (5).

Next, nuclei isolated from the three different silk gland samples were subjected to chemical cleavage with MPE-Fe(II), which had been shown to cleave linker DNA regions in chromatin with little sequence specificity (11). As shown in Fig. 3, the same fibH region as examined in Fig. 2 was sensitive to MPE-Fe(II) when the gene was transcriptionally actively (lanes 3-6) but was much less sensitive in temporarily and permanently inactive states (lanes 13-16 and 8-11, respectively). It was noticed that regular arrays of DNA fragments, approximately 0.35 to at least 1.4 kb, appeared when the two transcriptionally inactive chromatin were cleaved with MPE-Fe(II). These fragments were interpreted to be derived from arrays of nucleosomes.

Regular Appearance of DNase I-sensitive Subregions in the Transcriptionally Inactive fibH Chromatin—As shown in Fig. 2, at least six fragments of 12.6, 11.0, 9.3, 7.8, 6.7, and 5.4 kb were produced when the nuclei containing permanently and temporarily inactive fibH chromatin were digested with DNase I (lanes 10, 13, and 14). These bands were hardly seen when the nuclei containing transcriptionally active fibH chromatin (lanes 7 and 8) or the silk gland genomic DNA (lanes 3-5) were digested. Interestingly, sizes of these fragments corresponded to some of the bands produced by partial digestion of the genomic DNA with HindIII (lanes 15 and 16).

Most part of the second exon of fibH consists of repeats of DNA sequences coding for "crystalline" and "amorphous" region peptides of the silk fibroin, and a HindIII site is located within each of the sequences coding for the amorphous region (8). It is conceivable from these structural features that DNA sequences around the HindIII sites in the amorphous region-encoding sequences were preferentially cleaved with DNase I even in the transcriptionally inactive chromatin.

This suggestion was further supported from the following experiments. PSG or MSG nuclei were digested with increasing concentrations of DNase I and DNA purified from the nuclei was digested with HindIII. The DNA digests were electrophoresed and subjected to Southern blot hybridization with a DNA probe for the crystalline region (CR probe) or the amorphous region (AM probe) (Fig. 4, A and B). At least six HindIII fragments, designated a-f, were hybridized with both probes when the nuclei had not been digested with DNase I. When the nuclei were digested, bands hybridized with AM disappeared more quickly than bands hybridized with CR. Considering that the AM sequence is included in an ~50-bp AvalI-HinfI fragment in the amorphous region-encoding se-
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Fig. 1. Restriction sites relevant to this study and locations of probes in a genomic region containing fibH (A) and in a set of crystalline region-encoding sequences bordered with amorphous region-encoding sequences (B). Restriction sites are abbreviated as follows: AvaII (A), BglII (Bgl), EcoR I (E), EcoRV (V), HindIII (Hd), HinfI (H), MboI (M), PstI (P), and XhoI (X). Approximate positions (in kb) of restriction sites in parentheses are approximate. Probes were either subcloned DNA fragments; EH05 (0.5-kb EcoRI-HindIII fragment), P2 (0.6-kb PstI-PstI fragment), and PE1 (approximately 15-kb PstI-EcoRI fragment but having substantial deletions within the repetitive protein-encoding sequences that had occurred during cloning), or synthetic DNA fragments; CR and AM for crystalline and amorphous region-encoding sequences, respectively.

Fig. 2. Contrasting sensitivity of the second exon region in different silk gland nuclei against DNase I digestion. Nuclei (250 μg of DNA/ml) from PSG (lanes 6-8) or MSG (lanes 9-11) of the fifth instar larvae, or from PSG (lanes 12-14) of the fourth molting-stage larvae, or deproteinized DNA (250 μg/ml) from PSG (lanes 3-5) were digested with DNase I at concentrations indicated. DNAs purified were digested with PstI, electrophoresed on a 45-cm long 0.6% agarose gel (5 μg/lane), blotted and hybridized with the 32P-labeled PE1 probe. Samples in lanes 15 and 16 were B. mori genomic DNA from PSG that had been digested with HinfI to different extent followed by complete digestion with PstI. Size markers were mixtures of B. mori DNA digested with PstI, PstI + HindIII, and PstI + EcoR I (lane 1) or with PstI + BglII and PstI + XhoI (lane 2).

Fig. 3. Contrasting sensitivity of the second exon region in different silk gland nuclei against MPE-Fe(II) cleavage. Nuclei (150 μg of DNA/ml) from PSG (lanes 2-6) or MSG (lanes 7-11) of the fifth instar larvae, from PSG (lane 1) were treated with 0 or 20 μM (for nuclei) or 5 μM (for DNA) MPE-Fe(II) for different times as indicated. DNAs purified were digested with PstI and subjected to electrophoresis and blot hybridization with the 32P-labeled PE1 probe as in Fig. 2. Dots on the right of lanes 11 and 16 denote DNA fragments interpreted to represent nucleosomal ladders.

DNase I digestion than adjacent crystalline region-encoding sequences in the transcriptionally inactive chromatin.

DNase I- and MPE-Fe(II)-hypersensitive Sites in the 5'-Upstream Region of the fibH Chromatin in Different Silk Glands—In the previous study, two micrococcal nuclease-hypersensitive sites were detected in the fibH chromatin (5); one site (HS-1), which was also detected with DNase I in this study (Fig. 2), was located between the EcoRI site at the 3' end of the gene and the HindIII site (+17.5 kb) in the 3'-
flanking region, and the other site (HS-2) was located between the XhoI site in the intron and the PstI site near the 5'-end of the second exon of fibH. These two HS sites were detectable more clearly in the transcriptionally inactive chromatin, and thus they seemed to form boundaries of the inactive chromatin domain containing the second exon of fibH.

In this study, HS sites in the 5'-upstream region of the fibH in different silk gland nuclei were examined by the indirect end-labeling method using the EH05 probe located about 6 kb upstream of the transcription start point (Fig. 1). Nuclei isolated from different silk glands were digested partially with DNase I and DNAs purified from them were digested with EcoRI plus XhoI and subjected to Southern blot hybridization with 32P-labeled mixed oligodeoxynucleotide probes for sequences encoding crystalline region (CR) (A) or amorphous region (AM) (B). Sizes of HindIII fragments produced from the deproteinized B. mori DNA were: 4.3 (a), 2.1 (b), 1.6 (c), 1.5 (d), 1.4 (e; double bands), and 1.1 (f) kb.

Presence of HS-3 and -4 in the transcriptionally active chromatin was also shown in similar experiments with MPE-Fe(II). As shown in Fig. 6, two bands of about 5.7 and 4.1 kb, which corresponded to HS-3 and -4, respectively, appeared when the transcriptionally active fibH nuclei were treated with MPE-Fe(II) (lanes 3-6). On the other hand, these HS sites were undetectable in both permanently and temporarily inactive chromatin (lanes 8-11 and 13-16). Absence of these HS sites in the temporarily inactive chromatin was different from the results with DNase I (Fig. 5, lanes 8 and 9).

Next, DNase I hypersensitive sites in a further upstream region of the fibH were examined. Nuclei from different silk glands were partially digested with DNase I, and DNAs purified were digested with PstI and probed with 32P-labeled EH05. Fig. 7 shows that intensity of the 22-kb PstI-PstI band decreased and new bands of 15.5, 12.5, 4.3, and 3.9-3.6 kb appeared upon digestion with increasing concentrations of DNase I. These new bands corresponded to transcriptionally active chromatin (data not shown). Examination of the EcoRV-EcoRV region (-6.8 kb to +150 bp, Fig. 1) using the P2 probe (-546 to +61 bp) revealed that neither DNase I nor MPE-Fe(II) cleaved preferentially within the EH05 probe region (data not shown).

**High Resolution Analysis of DNase I Sensitivity of an Enhancer-Promoter Region of fibH Chromatin**—The cis-acting DNA sequence elements and the trans-acting protein factors required for the transcription of fibH have been studied by Suzuki and collaborators (12, 13) using cell-free transcription systems from PSG and MSG. It has been demonstrated from those studies that about 200-bp-long region upstream from the TATA box functions as the major enhancer region to yield maximum and tissue-specific transcription in vitro. However, details of in vivo chromatin structure of this region are unknown. Thus, we adopted the in vivo footprinting
procedure of Saluz and Jost (9) to examine DNase I sensitivity of a region containing the promoter and a part of enhancer sequences with high resolution. Nuclei from different silk glands were partially digested with DNase I, and DNAs purified from them were digested with EcoRI plus XhoI and subjected to linear amplification reactions by PCR with a single 32P-labeled 27-mer primer, i.e. nucleotide positions from +23 to -4 or +66 to +40 in the lower strand. Amplified DNA products were purified and resolved by sequencing gel electrophoresis. In these experiments, DNase I sensitivity of only the upper DNA strand was examined because 27-nucleotide-long sequences suitable as specific primers for the analysis of lower strand were not obtainable in this region.

In Fig. 8, DNase I sensitivities of the upper DNA strand are compared using the above two different primers. In these experiments, PSG nuclei containing the actively transcribed fibH were digested with lower concentrations of DNase I so that similar extent of digestion could be obtained for transcriptionally active and inactive chromatin. As shown in Fig. 8A, strongly enhanced cleavage sites at nucleotide positions -41 to -43 relative to the transcription start point, enhanced cleavage sites at -50, -56, and -57, and a number of protected
By looking at the image, the text reads as follows:

FIG. 8. In vivo DNase I footprinting for the upper DNA strand in the promoter-enhancer region of fibH in the nuclei of different silk glands. Digestions were performed at 20 °C for 5 min with increasing concentrations of DNase I (units/ml) as follows (left to right in each set): 0, 0.025, and 0.05 (A) or 0.03 and 0.05 (B) for deproteinized B. mori DNA; 0, 0.1, 0.5, 1, and 2 (A) or 0, 0.05, 0.1 and 0.5 (B) for the fifth instar PSG nuclei; 0, 0.1, 1, and 2 (A) or 0, 1, 2, and 3 (B) for the fifth instar MSG nuclei; and 0, 1, and 2 (A) or 0, 0.25, 1, and 2 (B) for the fourth molting-stage PSG nuclei. Lanes G and C are sequence markers as described under "Experimental Procedures." A PCR primer used for the linear amplification was an oligodeoxynucleotide complementary to the upper strand sequence from +23 to −4 (A) or from +66 to +40 (B). Numbers indicate nucleotide positions from the transcription start point. Protected (open triangle) and enhanced (filled triangle) cleavage sites, which were specific to the fifth instar PSG nuclei, are indicated at right.

sites against the cleavage at −31, −33, and between −65 and −114 were detected in the actively transcribed chromatin from the fifth instar PSG. Fig. 8B shows strongly enhanced cleavage sites at −42 and −45, enhanced cleavage sites at +8, −50, −65, −57, −66, and −79, clustered protected sites between −10 and −33, and scattered protected sites at −54, −65, and −74 for the transcriptionally active chromatin. Both temporarily and permanently inactive chromatin, from the fourth molting PSG and the fifth instar MSG, displayed generally similar cleavage profiles for the region examined.

**DISCUSSION**

The present study revealed that the three regions of fibH chromatin, i.e. 5′-upstream, enhancer-promoter, and the major protein-encoding regions, behaved differently in active, temporarily inactive, and permanently inactive states of transcription. Significance of these observations is discussed below.

**5′-Upstream Region**—HS sites around fibH detected in this and previous studies (5) are depicted in Fig. 9. It is noteworthy that HS-sites in the upstream region of fibH in PSG are generally similar, irrespective of its transcriptional states. However, when fibH is transcribed actively, HS-3 and -4 become more obvious and HS-minor is detectable, and furthermore, HS-6 and -7 are also sensitive to MPE-Fe(II). These results imply that the chromatin structure of the upstream region is a little more exposed in the transcriptionally active state than in the temporarily inactive state. On the other hand, an upstream HS site in the permanently inactive MSG chromatin is limited to HS-5. Thus, it seems that the chromatin structure of the upstream region in the temporarily inactive state is of transcriptionally poised (14), i.e. the chromatin conformation is maintained generally as in the active state so that active transcription of the gene can be readily resumed when the physiological constraint of the molting stage is over. This situation somewhat resembles that of the chromatin region encompassing the ovalbumin gene family in the hormone-withdrawn oviduct, where an upstream region
Fig. 9. Hypersensitive sites in chromatin domains for flanking and protein-encoding regions of fibH in different transcriptional states. Hypersensitive sites (HS) against DNase I (open arrow), micrococcal nuclease (filled arrow), or MPE-Fe(II) (striped arrow) are illustrated. Larger arrows mean that these sites were significantly more sensitive than other sites.

Fig. 10. Comparison of the results of in vivo DNase I footprinting and in vitro DNase I-protection assay. Protected (open triangle) and enhanced (filled triangle) cleavage sites specific to the fifth instar PSG nuclei, obtained from the in vivo footprinting experiments shown in Fig. 8, are indicated below the published upper strand sequence (28) of the region containing promoter and a part of enhancer sequences for fibH. Underlined symbols indicate prominently enhanced cleavage sites. Protected regions from DNase I digestion and enhanced cleavage sites in vitro in the presence of PSG nuclear extract (taken from Hui et al. (Ref. 24)) are shown above the sequence. Positions of TGTA repeats, TATA box and PCR primers used are shown with single and double underlines and arrows, respectively.

up to about -20 kb from the ovalbumin-related X gene remains to be DNase I-sensitive as in the estrogen-stimulated oviduct (15) and active gene transcription is resumed rapidly upon the secondary stimulation with estrogen (16). However, three DNase I HS sites at -0.8, -3.3, and -6.0 kb in the upstream region of the ovalbumin gene were inducible with estrogen and disappeared nearly completely upon withdrawal of the hormone (17).

Among the five PSG-specific HS sites in the upstream region of the fibH chromatin, HS-3, -4, -minor are different in nature from HS-6 and -7. The former three sites appear more prominently when fibH is transcribed actively, and thus they resemble to some extent hormone-inducible HS-sites such as above three sites of the ovalbumin gene chromatin, the -7.1-kb site in the chicken vitellogenin III gene chromatin (18) or the -2.5-kb site in the rat tyrosine aminotransferase gene chromatin (19). On the other hand, HS-6 and -7 resemble the -6.1-kb HS site in the chicken lysozyme gene chromatin (20) in that these sites are present constitutively in the cell types which have capability of expressing the gene. The -6.1-kb site of the lysozyme gene has been shown to have cell-type-specific enhancer activity by transfection and transient expression experiments (21). Although functions of HS-6 and -7 remain to be elucidated, they may be involved in the formation of PSG-specific, potentially active chromatin configuration of fibH.

Enhancer-Promoter Region—Analysis of DNase I sensitivity of this region at the sequence level revealed some features that were specific to the transcriptionally active state as shown in Fig. 10.2 The most notable one among them was the strongly enhanced cleavage of phosphodiester bonds between nucleotide positions -41 and -42 and also -42 and -43. These sites were located close to the protected region of -10

2 The nucleotide sequence shown in Fig. 10 was taken from Ref. 28 and is accessible by GenBank/EMBL accession number V00094 and J01027.
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to -33 that contained the TATA box. Thus, it is conceivable that the enhancement was due to torsional stress on this DNA region caused by interaction of the TATA-binding transcription factor IID complex with enhancer-binding activator proteins (22). Similar enhancement of DNase I digestion has been observed in the transthyretin gene chromatin of the mouse liver (23), where HS sites were detected between -39 and -44, which were located near a protected region between -19 and -35, when the gene was transcribed actively.

In vitro DNase I footprinting experiments on the enhancer-promoter region of fibH using linear DNA templates and the PSG nuclear extract have detected HS sites at -46, -50, -51, and -66 but no conspicuous enhancement of cleavage at -41, -42, and -43 (24). Thus, the latter HS sites seem to be formed as a consequence of local conformational change of chromatin in the nucleus. In Fig. 10, DNase I-protected regions detected in vitro in the presence of PSG nuclear extract (24) are also indicated. It has been suggested from the results of gel mobility shift assay that these protections are caused by binding of both silk gland-specific and ubiquitous factors (24). The present chromatin footprinting data are generally compatible with the in vitro results except for the presence of above strong HS sites and additional enhanced cleavage sites between -54 and -57 and at -79 in the chromatin.

Present results indicated that fine chromatin structure of the enhancer-promoter region of the transcriptionally active fibH was different from that of the temporarily inactive state, suggesting that association of protein factors to the TATA box and nearby enhancer-promoter sequences is a key step in the conversion from the transcriptionally poised to the active state.

Protein-encoding Region — A region containing the second exon of fibH was highly sensitive to DNase I or MPE-Fe(II) when it was transcribed actively in the PSG nuclei. Nucleosomal ladder-like DNA fragments were not produced when the region was cleaved with MPE-Fe(II), suggesting that this protection is highly sensitive to torsional stress on this DNA region caused by interaction of the TATA-binding transcription factor IID complex with enhancer-binding activator proteins (22).

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