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The Dynamics of Chromatin Condensation: Redistribution of Topoisomerase II in the 87A7 Heat Shock Locus during Induction and Recovery

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We have examined the in vivo sites of action for topoisomerases II in the 87A7 heat shock locus as a function of gene activity. When the *hsp70* genes are induced, there is a dramatic redistribution of topoisomerase II in the locus which parallels many of the observed alterations in chromatin structure. In addition to changes in the topoisomerase II distribution within the locus, we find topoisomerase II localized around the putative domain boundaries scs and scs'. During recovery, when the chromatin fiber of the locus recondenses, the major sites of action for topoisomerase II appear to be located within the two *hsp70* genes and in the intergenic spacer separating the two genes.

The chromatin fiber in quiescent regions of eukaryotic chromosomes is compacted more than 200- to 400-fold over that of B-form DNA. Several different levels of chromatin organization are thought to be required to achieve this high degree of compaction (for reviews, see references 19 and 36). The first is the beads-on-a-string array of nucleosome core particles. Each bead consists of ~145 bp of DNA wrapped around the histone octamer and a linker DNA segment of roughly 30 to 50 bp which separates adjacent octamers. The second level is the 30-nm fiber which is generated by the coiling of the beads-on-a-string array into a helical or solenoid-like structure (3, 28, 38, 39). Though still poorly understood, the next level of compaction is thought to be the segregation of the 30-nm fiber into a series of discrete and topologically independent higher-order domains (1, 2, 5, 14, 18, 23). The first two levels of organization account for a 40-fold compaction of the DNA, while another 5- to 10-fold is contributed by the last level (or levels) of organization.

When genetic units within one of these highly condensed domains are activated, the chromatin fiber unravels to give a more open or looped-out structure. The transitions in the topological organization of the chromatin fiber accompanying transcriptional activation can be readily visualized in salivary gland polytene chromosomes of *Drosophila melanogaster* (21, 29). For example, the *hsp70* heat shock genes at the 87A7 and 87C1 loci are expressed only at very low levels under normal growth conditions, and the chromatin fiber at each of these cytogenetic loci is tightly condensed in polytene chromosomes. When salivary glands are heat shocked, there is a rapid induction of the *hsp70* genes and the genes are transcribed at very high levels. Transcriptional activation is accompanied by the decondensation of the chromatin fiber and the formation of quite large puffs (25). In going from the uninduced to the fully induced state, each heat shock locus may undergo almost a 100-fold change in compaction. In the case of 87A7, for example, the chromatin fiber in the fully induced puff is condensed on average no more than two- to four-fold over naked B-form DNA, not much more than that expected for the beads-on-a-string arrangement of nucleosome core particles (31).

Such dramatic changes in the extent of compaction of the chromatin fiber are likely to be mediated by enzymes which are capable of altering the topology of DNA. In *D. melanogaster*, two different enzymes which might play a role in modulating the topology of the chromatin fiber before and/or after heat induction have been identified (for reviews, see references 17 and 37). The first is topoisomerase I. It catalyzes the relaxation of positively or negatively supercoiled DNA by acting as a swivel, transiently introducing and then resealing single-strand breaks. Topoisomerase I has been implicated in the topological alterations which occur when the *Drosophila* heat shock genes are expressed. Immunolocalization studies by Fleischmann et al. (4) have shown that high levels of topoisomerase I accumulate at the heat shock loci after induction, while little or no topoisomerase I is detected at these loci prior to induction. From UV cross-linking experiments, it would appear that much of the newly recruited topoisomerase I is associated with the transcribed sequences in each of these loci (7, 8). The preferential localization of topoisomerase I in transcribed DNA rather than in the flanking nontranscribed spacer sequences has been confirmed by mapping camptothecin-induced DNA scissions in several different heat shock loci (6).

A second enzyme which might play some role in modulating the topology of the chromatin fiber during heat shock and recovery is topoisomerase II. This enzyme catalyzes DNA relaxation by the transient formation of double-strand breaks in an ATP-dependent reaction (11, 17, 24). The role of this enzyme may be somewhat different from that of topoisomerase I. In contrast to topoisomerase I, which is recruited to the heat shock loci when they are transcriptionally activated, topoisomerase II can be detected in the heat shock loci in vivo even under conditions in which there is little or no expression of the heat shock genes (22, 33). In previous studies, Rowe et al. (22) found that heat shock altered the sites of action for topoisomerase II in the five *hsp70* genes at the cytogenetic loci 87A7 and 87C1. However, it is not possible to draw firm conclusions from this work, as the authors used probes located within the repeated

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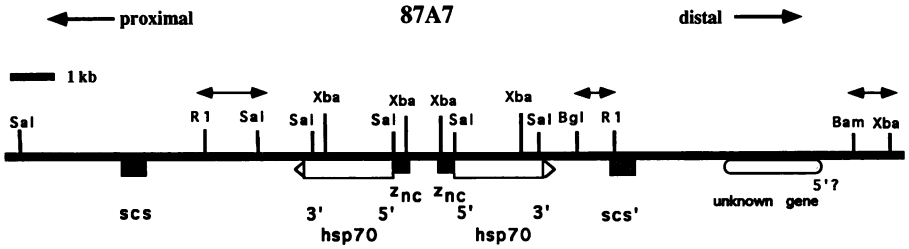

FIG. 1. Genomic map of the 87A7 heat shock locus and flanking regions. The locations of the two *hsp70* transcription units, the conserved 5′ nontranscribed sequences, Z_{nc}, at the beginning of each transcription unit, and scs and scs′ are indicated (from reference 31). Also shown are positions of the probes used for indirect end labeling. On the left, between the arrows is the *Eco*RI-*Sal*I probe used for visualizing the fragment pattern around the *hsp70* genes (after restriction with *Eco*RI) or around scs (after restriction with *Sal*I). Two probes, *Bgl*II-*Eco*RI and *Bam*HI-*Xba*I, are located to the right of the *hsp70* genes and are used to visualize scs′. The *Bgl*II-*Eco*RI probe can also be used to examine the topoisomerase II cleavage products in the distal *hsp70* gene.

hsp70 genes to analyze the topoisomerase II sites of action. With these probes, it is difficult to detect the changes in topoisomerase II distribution that occur within the genes after induction. In addition, since the genes are repeated in the genome, topoisomerase II moieties that map to DNA segments lying outside of the genes cannot be localized to specific sites in either 87A7 or 87C1. To obtain a clearer picture of the possible role of topoisomerase II in the topological reorganization of the chromatin fiber which accompanies gene activation and deactivation, we have examined the sites of action for the enzyme in the 87A7 heat shock locus in vivo before and after induction, using single-copy probes unique to this locus.

MATERIALS AND METHODS

Topoisiomerase II cleavage and mapping were performed essentially as described by Udvardy and Schedl (33). Briefly, 0.8 to 1.5 liters of *Drosophila* KC tissue culture cells grown at 25°C was divided into equal portions and then allowed to continue growing at this temperature for 1 to 2 h. The different portions were then heat shocked at 37°C, allowed to recover at 25°C, and treated or not treated with VM-26 as indicated in the figure legends. Subsequently, the cells were harvested by centrifugation and rapidly resuspended in 1 to 3 ml of buffer containing 60 mM NaCl, 15 mM Tris-Cl (pH 7.5), and 250 mM sucrose. Double-strand breaks were then induced by the addition of sodium dodecyl sulfate (SDS) to 1%. After a ~10-min incubation at room temperature, EDTA (final concentration of 25 mM) and proteinase K (100 μg/ml) were added, and the lysate was incubated either at 65°C for several hours or at 37°C overnight. After three phenol extractions, the DNA was ethanol precipitated three times and resuspended in 10 mM Tris-Cl (pH 7.5)–1 mM EDTA for restriction enzyme digestion. The restriction digests and probes are as described in the figure legends and text. Micrococcal nuclease and S1 nuclease digests of KC cell nuclei were as described by Han et al. (9).

The double-stranded DNA cleavage products formed by topoisomerase II have an enzyme moiety covalently linked to the 5′ terminus of each strand. We used the procedures described by Udvardy and Schedl (33) to determine whether a protein moiety is attached to the VM-26-induced cleavage products observed in the 87A7 heat shock locus both before (reference 33 and data not shown) and after (not shown) induction. We found that all of the major VM-26-induced cleavage products appeared to have a covalently attached protein moiety (e.g., pelleting with the DNA in CsCl or sucrose gradients, resistance to 1 to 5% SDS at 65°C for 2 to 6 h, and sensitivity to proteinase K digestion), as judged by a gel retardation assay (using an agarose gel with 1% SDS in the buffer).

RESULTS

Chromatin organization of the 87A7 heat shock locus. Shown in Fig. 1 is a map of the 87A7 heat shock locus and its flanking DNA segments. This locus contains two divergently transcribed *hsp70* genes separated by a small intergenic spacer. Studies of the chromatin organization of the locus in non-heat-shocked cells indicate that there is an ordered nucleosome array extending across the intergenic spacer (10, 32). The 5′ end of each gene is hypersensitive to nuclease cleavage, while the genes and the immediately flanking 3′ spacer segments are comparatively resistant to nuclease attack and give only a weak nucleosomal fragment pattern. This chromatin organization changes dramatically after induction (16). The nucleosomes in the intergenic spacer shift in position, while the genes become extremely nuclease sensitive (9, 32).

The 87A7 heat shock locus is flanked on either side by two unusual chromatin structures, scs and scs′, which may correspond to the proximal and distal ends, respectively, of the chromomere (31). These putative domain boundaries (12, 13) are defined by a pair of nuclease-hypersensitive sites surrounding a small nuclease-resistant core of ~200 to 350 bp. scs is ~4 kb from the 3′ end of the proximal *hsp70* gene, while scs′ is ~2 kb from the 3′ end of the distal gene. Although far removed from the transcribed sequences, the nuclease cutting pattern at both scs and scs′ is altered by heat induction of the *hsp70* genes.

Heat shock alters the distribution of topoisomerase II in the 87A7 heat shock locus. The topoisomerization reactions catalyzed by topoisomerase II involve the transient formation of a covalent protein-DNA complex (24). The sites of action for the enzyme can be determined by taking advantage of this intermediate in the catalytic cycle; addition of denaturants traps the complex, generating a double-strand scission with a protein moiety covalently attached to the 5′ end of each strand (15, 24). Since cleavage is relatively inefficient, a drug which stabilizes the covalent intermediate, VM-26, is used to stimulate the reaction in vivo (17, 22, 33). In the experiment presented in Fig. 2, either control or heat-shocked *Drosophila* KC tissue culture cells were treated with increasing concentrations of VM-26 for 40 min. The distribution of VM-26-induced cleavage products in the DNA segment which contains the 87A7 *hsp70* genes was

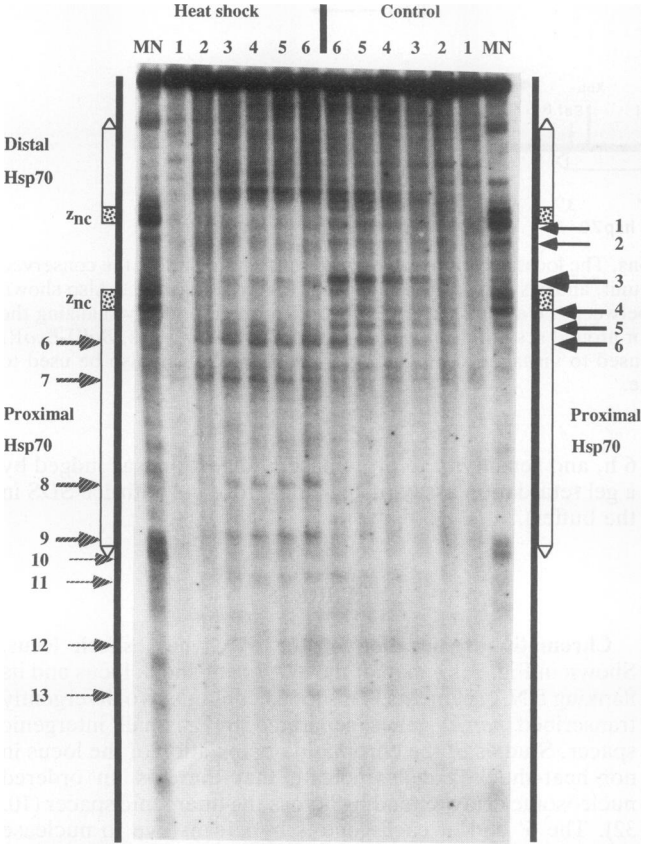

FIG. 2. Topoisomerase II cleavage around the 87A7 *hsp70* genes before and after induction. Approximately 1 liter of *Drosophila* KC tissue culture cells grown at 25°C was split into 12 equal aliquots in spinner flasks. Six of these aliquots were incubated 25°C, while the other six were shifted to 37°C for 20 min. The aliquots at 25 and 37°C were then treated with no VM-26 or increasing concentrations of this drug for 40 min, harvested by centrifugation, and processed as described previously (33). The DNA samples isolated from each aliquot were restricted with *Eco*RI and following electrophoresis and blotting probed with an *Eco*RI-*Sal*I restriction fragment from the proximal side of 87A7. This displays the topoisomerase II cleavage products in the 10-kb 87A7 *Eco*RI fragment reading through the proximal *hsp70* gene into the intergenic spacer and the distal *hsp70* gene as indicated in the diagram. MN, micrococcal nuclease. For control and heat-shocked cells, VM-26 concentrations were as follows: lane 1, no drug; lane 2, 1 μg/ml; lane 3, 2.5 μg/ml; lane 4, 5 μg/ml; lane 5, 10 μg/ml; and lane 6, 15 μg/ml. Also indicated in the diagram are numbers of the topoisomerase II cleavage sites in the proximal gene and the intergenic spacer before and after induction. We also established that there is a covalent linkage of protein moieties to the VM-26-induced cleavage products from control and heat-shocked cells (not shown), using the procedures described previously (33). Band numbers are indicated in the diagram and discussed in the text. Band 7 is the lower band of a doublet. This doublet is found in both control and heat-shocked cells. However, the fragments are detected even without VM-26, and with the exception of band 7 in heat-shocked cells, cleavage is not greatly stimulated by drug. Hence, most of the fragments in this doublet are probably not generated by topoisomerase II.

then determined by indirect end labeling using an *Eco*RI-*Sal*I probe (Fig. 1).

In control tissue culture cells, most of the major topoisomerase II cleavage products are clustered around the 5′ ends of the two *hsp70* genes (Fig. 2) (33). In the intergenic spacer separating the two genes, there is one major topoisomerase site (site 3) which maps to an internucleosomal linker one nucleosome upstream of the 5′ end of the proximal *hsp70* gene and two minor sites (sites 2 and 1) which map to internucleosomal linkers on the distal side of the spacer. At the 5′ end of each *hsp70* transcription unit there are three major sites; one (site 4) is close to the start site, and the other two (sites 5 and 6) are spaced at nucleosome-length intervals downstream (see Fig. 8A). This cleavage pattern changes dramatically after induction (Fig. 2 and 8A) (22). First, there is a substantial reduction in topoisomerase cleavage in the intergenic spacer. Second, of the three major sites at the beginning of the transcription unit, only the one farthest from the 5′ end (site 6) is still a strong target for the enzyme after induction. Third, cleavage within the two *hsp70* genes is substantially enhanced by induction. In the proximal gene, three prominent bands (bands 7 to 9) appear, and a similar set of bands is observed in the distal gene (with use of a probe from the distal side [not shown]). Finally, cleavage at several minor bands at the 3′ end of the gene and in the 3′ flanking spacer (bands 10 to 13) increases after induction. Similar results were reported by Rowe et al. (22).

We also examined the distribution of topoisomerase II in other loci before and after heat shock (unpublished results). Alterations similar to those reported here were found around the *hsp70* and αβα genes in the 87C heat shock locus and around the *hsp83* gene in the 63BC heat shock locus. In contrast to these heat shock loci, no major heat-induced changes in the cleavage pattern were evident in the *rudimentary* locus, in a tRNA gene cluster at 90BC that contains an scs-like element (13, 33), or in the *Bithorax* complex.

Relocalization of topoisomerase II during recovery. When heat-shocked *Drosophila* cells are returned to 25°C, transcription of the *hsp70* genes gradually diminishes and the preinduced chromatin organization of the 87A7 locus is reestablished. Like induction, the recovery process involves extensive alterations in the topology of the chromatin fiber, and it was of interest to examine the localization of topoisomerase II. For this purpose, heat-shocked cells were allowed to recover at 25°C for different times before being treated with VM-26. In the experiment shown in Fig. 3, the initial phase of the recovery process is examined. Throughout this period, there is a high level of cleavage at heat shock site 6 near the 5′ end of the proximal gene (and at the equivalent site in the distal gene [not shown]). While the level of fragment 6 remains high, the yield of other cleavage products changes as the time of recovery lengthens. Cleavage at the three heat shock sites (sites 7 to 9) within the gene decreases, while there is an increase in the yield of preinduced fragments from the intergenic spacer (particularly fragment 3) and to a lesser extent from the beginning of the transcription unit (fragments 4 and 5).

Localization of topoisomerase II around scs. The putative domain boundary on the proximal side of the 87A7 locus, scs, is located downstream from the proximal *hsp70* gene (Fig. 1). The pattern of topoisomerase II cleavage around this unusual chromatin structure can be visualized by probing *Sal*I-restricted DNA from VM-26-treated cells with the *Eco*RI-*Sal*I probe used above. The autoradiogram in Fig. 4 shows the distribution of topoisomerase II before and after heat shock, using the same DNA samples as in Fig. 2.

Prior to induction, the strongest topoisomerase II cleavage products are a set of three bands (bands 22 to 24) in the region just beyond scs. Each of these bands is aligned with a micrococcal nuclease cleavage product in chromatin digests (Fig. 4), and the three bands are spaced at nucleosome length

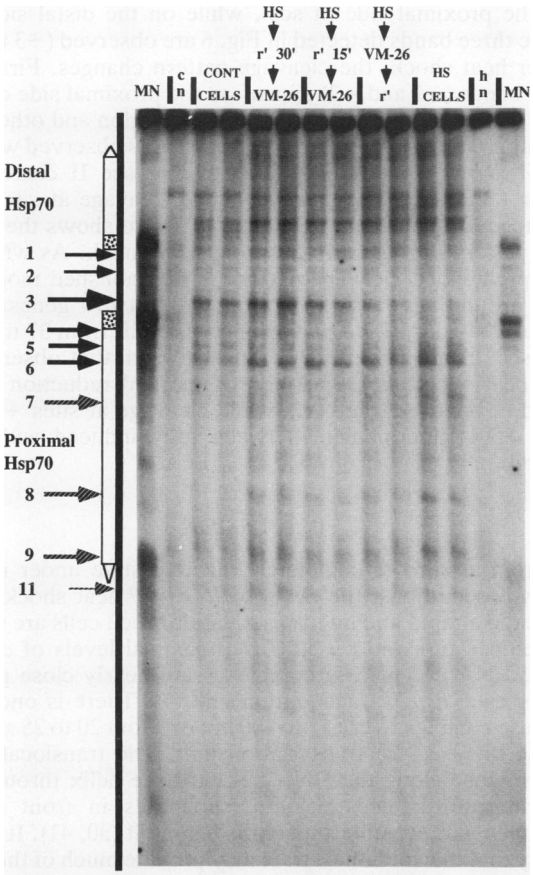

FIG. 3. Topoisomerase II cleavage products around the 87A7 heat shock genes during recovery at 25°C. Approximately 1 liter of *Drosophila* KC tissue culture cells was divided into 12 equal portions. The first two samples (CONT CELLS) were treated with VM-26 at 5 μg/ml (right lane) or 10 μg/ml (left lane) for 30 min at 25°C. The second set of samples (HS CELLS) was heat shocked at 37°C for 30 min and then treated with VM-26 at 5 μg/ml (right lane) or 10 μg/ml for 30 min at the elevated temperature. The third set of samples (HS→VM-26→r′) was heat shocked at 37°C for 60 min, VM-26 (5 μg/ml [right lane] and 10 μg/ml [left lane]) was then added, and the cells were immediately shifted back to 25°C for 30 min. The fourth set (HS→r′ 15′→VM-26) was heat shocked for 60 min, shifted back to 25°C for 15 min, and then incubated with VM-26 (5 or 10 μg/ml) for an additional 30 min at 25°C. The fifth set (HS→r′ 30′→VM-26) was heat shocked for 60 min, shifted back to 25°C for 30 min, and then incubated with VM-26 (5 or 10 μg/ml) for another 30 min at the low temperature. Samples cn and hn were prepared from cells incubated without drug at either 25 or 37°C. DNA was prepared from cells in each aliquot and subsequently analyzed as described in the legend to Fig. 2 and in reference 33. Lanes labeled MN are micrococcal nuclease digests of chromatin.

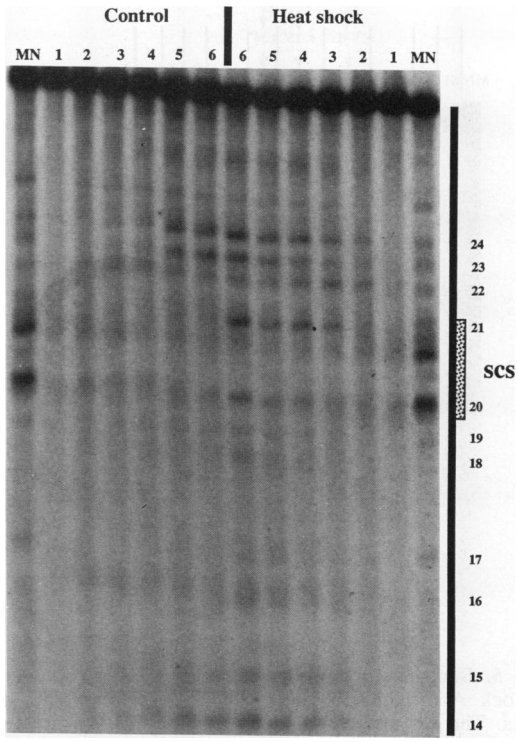

FIG. 4. Topoisomerase II cleavage at scs after heat induction. The experiment displays the VM-26-induced topoisomerase II cleavage products around scs in control and heat-shocked KC tissue culture. DNA samples 1 to 6 from the drug-treated control and heat-shocked cells were the same as those described for Fig. 2. However, instead of being restricted with *Eco*RI, the DNA was cut with *Sal*I. The filters were probed with the same *Eco*RI-*Sal*I fragment as in Fig. 2. MN, DNA prepared from a micrococcal nuclease digest of chromatin. We also established that the VM-26-induced cleavage products from control and heat-shocked cells around scs have a covalently linked protein moiety (not shown), using the procedures described previously (33). Cleavage around scs prior to induction appears to be somewhat less efficient than at the major preinduced sites around the two 87A7 *hsp70* genes. The three fragments from just beyond scs (fragments 22 to 24) are strongly labeled only at the highest drug concentrations (fifth and sixth lanes in Fig. 3). By comparison, band 3 in the intergenic spacer and the cluster of bands at the 5′ end of the *hsp70* genes are more strongly labeled at lower concentrations of VM-26 (fourth and fifth lanes in Fig. 3).

intervals. Previous studies indicate that heat shock alters the scs chromatin structure and that major changes in the nuclease cleavage pattern, particularly in the nuclease-hypersensitive regions, are observed. As illustrated in Fig. 4, heat shock also alters the topoisomerase II cleavage pattern. The most obvious change is the appearance of two new strongly labeled bands, 20 and 21, in the nuclease-hypersensitive regions which define the scs nucleoprotein structure. Other minor changes in the topoisomerase II distribution are also evident (e.g., the new bands 18 and 19).

During recovery from heat shock, the preinduced topoisomerase II cleavage pattern is reestablished more rapidly around scs than it is around the two *hsp70* genes (see above). As shown in Fig. 5, when cells are treated with VM-26 and then returned to 25°C for 30 min, the heat shock topoisomerase II cleavage products (products 20 and 21) in the scs nuclease-hypersensitive regions are still evident. However, when cells are treated with VM-26 at 15 or 30 min after the shiftdown (and incubated for an additional 30 min), these heat-induced topoisomerase II cleavage products disappear and the distribution of fragments resembles that observed prior to induction.

Localization of topoisomerase II around scs′. (i) scs′ reading toward 87A8. scs′, the putative domain boundary on the distal side of 87A7, is located just beyond the 3′ end of the distal *hsp70* gene. As indicated in Fig. 1, scs′ and a nearby transcription unit can be displayed by probing *Bgl*II digests with a *Bgl*II-*Eco*RI fragment. Figure 6 shows the effects of heat induction on the localization of topoisomerase II in the

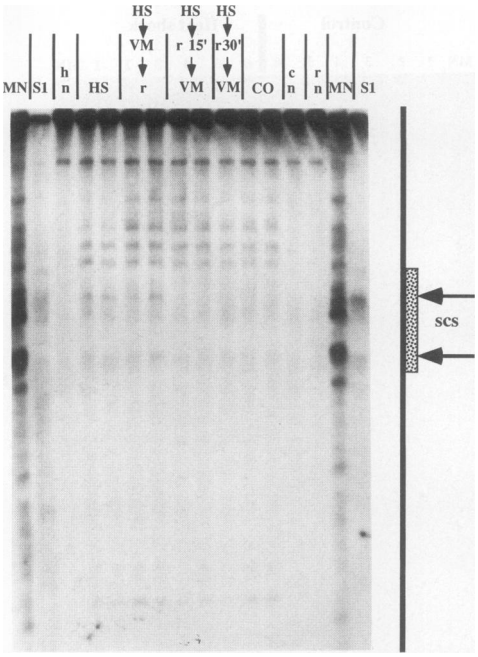

FIG. 5. Redistribution of topoisomerase II during recovery from heat shock. Approximately 1 liter of KC cells was split into 12 equal portions. The set of DNA samples labeled CO was treated with increasing amounts of VM-26 (5 or 10 μg/ml) for 40 min at 25°C. The two DNA samples labeled HS were first shifted to 37°C for 20 min and then treated with either 5 or 10 μg of VM-26 per ml for 40 min at the elevated temperature. The two samples labeled HS→VM→r were first heat shocked for 60 min. VM-26 (5 or 10 μg/ml) was added, and cells were allowed to recover at 25°C for 30 min. The two samples labeled HS→r 15′→VM were heat shocked for 60 min and then shifted back to 25°C for 15 min. VM-26 (5 or 10 μg/ml) was added, and the cells were allowed to recover for an additional 30 min. The sample labeled HS→r30′→VM was heat shocked for 60 min and then returned to 25°C for 30 min. VM-26 (10 μg/ml) was added, and the cells were incubated at 25°C for an additional 30 min. DNA samples labeled cn, hn, and rn were prepared from control cells, VM-26-treated heat-shocked cells, and untreated heat-shocked cells which were allowed to recover for 90 min. MN, micrococcal nuclease digest of chromatin; S1, S1 nuclease digest of chromatin. The S1 sample on the left was prepared from heat-shocked cells, while the sample on the right was prepared from control cells.

region to the distal side of 87A7. Prior to induction, there are three relatively prominent topoisomerase II cleavage products (+3, +4, and +5) located to the distal edge of scs′. The first, +3, is in the distal scs′ nuclease-hypersensitive site, while the other two cleavage products map in the region beyond the hypersensitive site in linker DNA segments separating adjacent nucleosomes. After induction, the relative yield of these three cleavage products changes: the yield of fragment +4 drops, while both +3 and +5 become stronger.

(ii) scs′ reading toward 87A7. The probe used for the experiment in Fig. 6 is located very close to scs′, making it difficult to visualize topoisomerase II cleavage products from the proximal side of this putative boundary. This problem can be overcome by cleaving the chromosomal DNA with *Xba* and then probing with a *Bam-Xba* fragment located just beyond the nearby transcription unit (Fig. 1). In Fig. 7, the region containing scs′ has been enlarged. Prior to heat induction, there is little or no topoisomerase II cleavage on the proximal side of scs′, while on the distal side, the same three bands detected in Fig. 6 are observed (+3 to +5). After heat shock, the cleavage pattern changes. First, two new bands (+1 and +2) appear on the proximal side of scs′, one (+2) from the scs′ hypersensitive region and other (+1) about 200 bp farther upstream. Second, as observed with the *Bgl*II-*Eco*RI probe (Fig. 6), topoisomerase II cleavage at sites +3 and +5 is enhanced, while cleavage at site +4 is reduced. The autoradiogram in Fig. 7 also shows the cleavage pattern during recovery from heat shock. As with scs, the preinduced cleavage pattern is reestablished more rapidly around scs′ than it is around the *hsp70* genes. Even when VM-26 is added just prior to the shift from 37 to 25°C, the scs′ cleavage pattern is different from that observed in the heat shock samples: there is marked reduction in the yield of bands +1 and +2, while cleavage at sites +3, +4, and +5 is intermediate between fully induced and uninduced.

DISCUSSION

From an almost completely inactive state under normal growth conditions, the *Drosophila hsp70* heat shock genes undergo a rapid and intense activation when cells are shifted to temperatures above 30°C. At maximal levels of expression, RNA polymerase complexes are nearly close packed along each *hsp70* transcription unit (7). There is one polymerase complex every 75 to 100 bp, or about 20 to 25 arrayed along the ~2.2-kb transcription unit. The translocation of polymerase along the DNA helix (or the helix through the polymerase) induces positive supercoils in front of the complex and negative supercoils behind it (30, 41). It seems likely that this torsional strain may provide much of the force driving the decondensation of the 87A7 chromatin domain after heat induction. The torsional strain generated by polymerase translocation may be further compounded by the fact that the *hsp70* gene is not a linear DNA duplex but instead is wrapped around 11 consecutive nucleosomal particles which are themselves coiled into a 30-nm fiber. How the polymerase complex actually circumnavigates the DNA wound around each core particle is not entirely clear (27, 36); however, it would not be unreasonable to suppose that this process also generates torsional strain and promotes the unfolding of the chromatin domain.

In the following discussion, we have attempted to relate the observed changes in the 87A7 topoisomerase II sites of action during heat shock and recovery to the alterations in chromatin structure (9, 10, 16, 20, 26, 31, 32) and the transitions in the topology of the chromatin fiber (25, 29, 31) that are known to occur in this locus when the *hsp70* genes are turned on and off. In doing so, it must be emphasized that we have made a number of key assumptions. The first is that the sites of action for topoisomerase II in vivo are largely dictated by accessibility (e.g., by nucleosome position and/or nonhistone proteins associated with the DNA). That this is likely to be a valid assumption has been reasonably well documented in previous studies on topoisomerase II cleavage in naked DNA (15, 35), in chromatin (reference 34 and unpublished data), and in vivo (22, 33). The second is that VM-26 does not greatly perturb the distribution of topoisomerase II in vivo. While previous studies on the naked DNA (35), chromatin (34), and in vivo (22, 33) sites of action of topoisomerase II would be consistent with this assumption, we have no independent means of verifying it. The third, and most critical, is that topoisomerase II plays an active role in facilitating some of the transitions in the

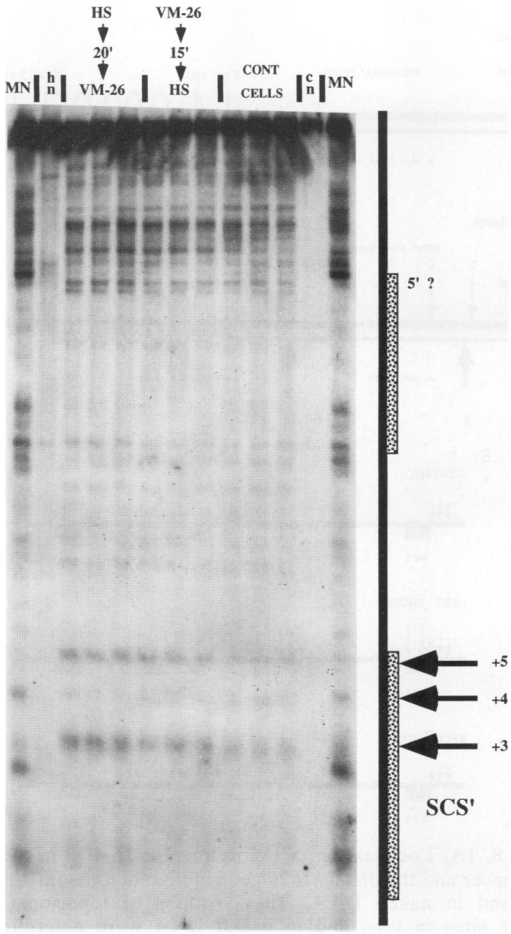

FIG. 6. Localization of topoisomerase II around scs': reading from 87A7 toward the telomere. Approximately 1 liter of KC tissue culture cells was divided into 12 equal portions. The samples in the three lanes labeled CONT CELLS were from cells treated with increasing amounts of VM-26 (right to left, 5, 10, and 15 μg/ml) at 25°C for 40 min. The samples in the three lanes labeled VM-26→15'→HS were from tissue culture cells treated with increasing amounts of VM-26 (right to left, 5, 10, and 15 μg/ml) at 25°C for 15 min. The cells were then shifted to 37°C for 60 min. The samples labeled HS→20'→VM-26 were first heat shocked for 20 min. Increasing amounts of VM-26 (right to left, 5, 10, and 15 μg/ml) were then added, and the cells were incubated for an additional 40 min at 37°C. The samples labeled cn and hn were from control and heat-shocked tissue culture cells, respectively, which were not treated with VM-26. DNA was prepared from each of these portions and restricted with *Bgl*II. The lanes labeled MN are DNA samples from micrococcal nuclease digests of KC cell chromatin restricted with *Bgl*II. After gel electrophoresis and blotting, the nitrocellulose filters were probed with a *Bgl*II-*Eco*RI fragment from the distal side of the 87A7 locus. This displays the topoisomerase II cleavage products reading from scs' toward the telomere. The positions of scs' and a nearby transcription unit (and its likely orientation) are indicated. Though the direction of transcription of this nearby gene has not been established, a nuclease-hypersensitive region which might correspond to the 5' end has been identified (see Fig. 1). As can be seen in the autoradiogram, the DNA segment around the (presumed) 5' end has several topoisomerase II cleavage products prior to induction. These fragments are derived from sites located both within and upstream of the transcription unit. As for the *hsp70* genes, the cleavage pattern in this nearby transcription unit changes after induction. The most obvious is a substantial increase in the yield of a band located upstream of the transcription unit. In this respect, it may be interesting that cleavage in the intergenic spacer separating the two 87A7 *hsp70* genes decreases rather than increases

topology of the chromatin fiber which occur when the heat shock genes are turned on or off and 87A7 locus puffs or regresses. A corollary is that the topoisomerase II sites of action observed during induction and recovery are relevant to the supposed role of the enzyme in the decondensation and condensation of the locus. Although we would argue that these assumptions are reasonable, they must necessarily temper the interpretation of how topoisomerase II might function in chromatin decondensation and condensation that is outlined in the following sections.

Induction. (i) Intergenic spacer. Previous studies have suggested that the intergenic spacer separating the two *hsp70* genes contains six aligned nucleosome core particles. The linker-to-linker distances of these nucleosomal units range from only 160 bp for the nucleosomes in the middle of the spacer to more than 250 bp for the nucleosomes on each edge of the spacer (32). Prior to induction, two of the major topoisomerase II sites are located in the linkers defining the larger nucleosomal units at each edge of the spacer (sites 1 and 3 in Fig. 8A), while the third site (site 2) is in a linker separating the second and third nucleosomes on the distal side of the spacer (33). This array of aligned core particles appears to be altered by heat shock, and the nucleosomes in the spacer appear to collapse into a much more tightly condensed complex (32). The two outer nucleosomes shift into the spacer, as judged by the appearance of new micrococcal nuclease cleavage products located just upstream of the *Xba*I sites in each Z_{nc} element and by the almost complete disappearance of micrococcal nuclease fragments derived from the linkers corresponding to topoisomerase II sites 1 and 3 (Fig. 8A) (32). Micrococcal cleavage at the other internucleosomal linkers in the intergenic spacer is also greatly reduced. At least two factors could contribute to these changes in nucleosome position. First, the outer nucleosomes might be displaced by the association of nonhistone proteins (e.g., heat shock factor) with the *hsp70* promoter (Z_{nc}) after induction. Second, torsional strain generated by the divergent transcription of the two *hsp70* genes may supertwist the intergenic spacer, causing the nucleosome array to condense (41). Consistent with the idea that the nucleosome array may be supertwisted by downstream transcription, the intergenic spacer becomes slightly S1 nuclease sensitive after heat induction (9). Whatever the mechanism, the rearrangement of nucleosome particles in the spacer should make the normal targets for topoisomerase II, the internucleosomal linkers, much less accessible. Indeed, there is little or no VM-26-induced cleavage in the spacer after induction (Fig. 8A). Of course, as a consequence of this limited accessibility, topoisomerase II would not be able to directly release any torsional strain in the spacer generated by the divergent transcription of the two *hsp70* genes. (There is also very little topoisomerase I in the spacer after induction [unpublished data].)

(ii) *hsp70* gene. The perturbations in *hsp70* gene chromatin after transcriptional activation have been well documented (16, 32, 40); the nucleosomal ladder observed prior to heat

when the heat shock genes are activated. This nearby transcription unit does not appear to be a heat shock gene and, like many other genes, may be turned off at elevated temperatures. If this is the case, the redistribution of topoisomerase II observed here may reflect a reorganization of the chromatin fiber as the gene is shut down. Alternatively, the changes could be a consequence of torsional strain propagated along the chromatin fiber from the highly active *hsp70* genes.

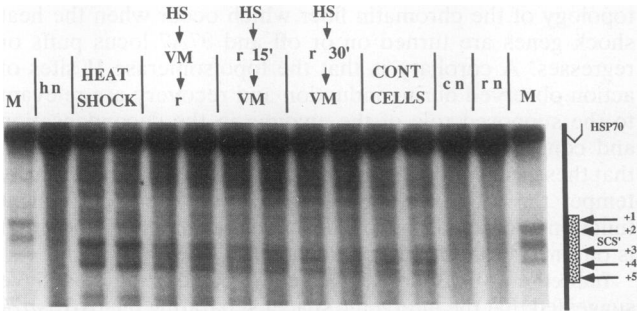

FIG. 7. Redistribution of topoisomerase II around scs' during heat shock and recovery: reading toward 87A7. Approximately 1.2 liters of KC tissue cells was divided into 13 equal portions. The two KC cells samples labeled CONT CELLS were treated with VM-26 at 10 μg/ml (right lane) or 20 μg/ml (left lane) at 25°C for 60 min. The two samples labeled HEAT SHOCK were shifted to 37°C for 20 min and then treated with VM-26 at 10 μg/ml (right lane) or 20 μg/ml (left lane) for 40 min. The two KC cell samples labeled HS→VM→r were heat shocked at 37°C for 60 min, VM-26 (10 μg/ml [right lane] or 20 μg/ml [left lane]) was added, and the cells were shifted back to 25°C for 40 min. The two KC cell samples labeled HS→r 15'→VM were first heat shocked for 60 min at 37°C and then allowed to recover at 25°C for 15 min. VM-26 (10 or 20 μg/ml) was then added, and the cells were incubated for an additional 40 min at 25°C. The two KC cell samples labeled HS→r 30'→VM were heat shocked for 60 min at 37°C and then returned to 25°C for 30 min. VM-26 (10 or 20 μg/ml) was added, and incubation continued for an additional 40 min at 25°C. The lanes labeled cn, hn, and rn are DNA samples prepared from KC cells which were not treated with VM-26. cn was prepared from cells grown at 25°C, hn was prepared from cells heat shocked for 60 min, and rn was prepared from heat-shocked cells which were allowed to recover for 70 min. DNA isolated from each of these samples was restricted with *Xba*I and analyzed by gel electrophoresis and blotting to nitrocellulose filters. The filter was probed with an *Xba*I-*Bam*HI restriction fragment located upstream of the transcription unit shown in Fig. 6. scs' is located ~7.0 kb from the *Xba*I restriction site. To visualize the cleavage products around scs', only this region of the autoradiogram is shown. The DNA in lanes labeled M is from a micrococcal nuclease digest of KC cells restricted with *Xba*I.

shock disappears, and the entire transcription unit plus the immediately flanking 3' spacer become extremely sensitive to nuclease cleavage. Although this dramatic increase in nuclease sensitivity indicates that the nucleoprotein organization of gene DNA is severely disrupted by transcription, the sites of action for topoisomerase II are not equivalent to those observed in naked DNA (see comparison in Fig. 8A). What factors limit accessibility to topoisomerase II is not entirely clear. While cross-linking experiments indicate that histones remain associated with *hsp70* gene DNA after induction (20, 26), it has been argued that the core particles unfold or split into subnucleosomal units, breaking or altering many of the normal nucleosome-DNA contacts during transcription (5, 27). We do not, however, observe multiple VM-26-induced cleavage products distributed at subnucleosomal or even nucleosomal lengths across the transcription unit. Hence, if core particle splitting actually occurs, it apparently does not expose DNA sequences of sufficient length for interaction with topoisomerase II. In fact, accessibility after induction is surprisingly limited, and there are only four prominent fragments. One (fragment 6; Fig. 8A) is observed prior to induction (and maps to an internucleosomal linker sequence). The other sites are spaced at intervals of 400 bp (fragments 6 and 7), 900 bp (fragments 7 and 8), and

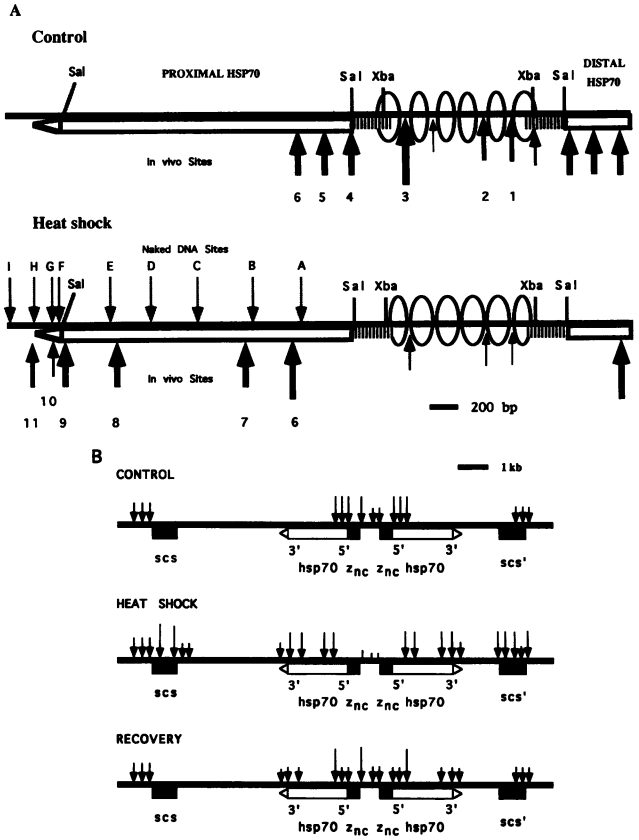

FIG. 8. (A) Localization of topoisomerase II sites in the intergenic spacer and the distal *hsp70* gene in vivo before and after heat shock and in naked DNA. The positions of topoisomerase II cleavage sites in vivo and in naked DNA were determined by agarose gel electrophoresis, blotting to nitrocellulose filters, and indirect end labeling (Fig. 2 and 3) (33–35). In naked DNA, the major sites for topoisomerase II cleavage in the 87A7 *Eco*RI fragment map to the intergenic spacer separating the two genes (35). There are several moderately strong sites at the 3' end of each gene, while there are only minor sites in the genes. However, none of these primary naked DNA sites appears to be cleaved by the enzyme in tissue culture cells grown at 25°C (33). Instead, the enzyme utilizes only sites that are in an exposed configuration in chromatin even though these sites often correspond to rather poor targets in naked DNA. While topoisomerase sites of action in vivo appear to be largely dictated by chromatin structure when the *hsp70* genes are off, this might change after induction. The very high levels of expression cause quite extensive alterations in chromatin structure, and the entire *hsp70* transcription unit becomes extremely sensitive to nuclease attack (16). For this reason, we have compared the sites of topoisomerase II cleavage in the proximal *hsp70* gene in naked DNA and in heat-shocked tissue culture cells. None of the four strong topoisomerase II sites (sites 6 to 9) in the *hsp70* gene from heat-shocked cells corresponds to a primary naked DNA site. In fact, with the exception of a minor fragment located just upstream of band 6 (see Fig. 2 and 3) which corresponds to arrow A, the primary naked DNA sites in the *hsp70* gene do not appear to be targets for topoisomerase II in heat-shocked cells. In the 3' flanking spacer, just downstream from the *Sal*I site (Fig. 2 and 3), there are four moderately strong naked DNA sites (F to I). Of these, only G (site 10) and H (site 11) are cleaved in vivo, and both are relatively minor sites. (B) Redistribution of topoisomerase II in the 87A7 heat shock locus during induction and recovery. Shown is a summary of the distribution of topoisomerase II in the 87A7 heat shock locus. Control, cleavage pattern before heat shock; heat shock, cleavage pattern after heat induction; recovery, cleavage pattern observed in samples shifted down to 25°C for 15 to 30 min. The size of each arrow indicates the relative yield of each cleavage product.

400 bp (fragments 8 and 9). This spacing could potentially correspond to di-, penta, and dinucleosomal units, respectively. Such units might be generated by the uncoiling of the 30-nm fiber into substructures which are still somewhat more complex than a simple beads-on-a-string nucleosome array. Alternatively, topoisomerase II cleavage could be limited by other factors such as polymerase molecules paused transiently at specific sites in the *hsp70* genes.

(iii) scs and scs'. The unusual chromatin structures scs and scs' are altered following heat shock. Since both elements are located well beyond the DNA segments whose chromatin structure is directly disrupted by the transcription of the two *hsp70* genes (9, 32), it was suggested that the perturbations observed in scs and scs' might be induced indirectly by torsional strain, propagated along the chromatin fiber from the two divergently transcribed genes (31). This led to the idea that these unusual chromatin structures might delimit the primary domain of decondensation for the 87A7 chromomere. Consistent with this possibility, scs and scs' appear to be located at or near the proximal and distal edges, respectively, of the 87A7 puff.

The results reported here provide additional support for the suggestion that scs and scs' may facilitate the long-range decondensation of the 87A7 chromatin fiber. Both elements are targets for topoisomerase II action after induction (Fig. 8B). In the case of scs, topoisomerase II cleavage is observed in the nuclease-hypersensitive regions to either side of the nuclease-resistant core after but not before heat shock. In the case of scs', two new topoisomerase II cleavage products appear on the proximal side of the nuclease-resistant core, while on the distal side there is a change in the relative yield of three cleavage products (Fig. 8B). These changes in the distribution of topoisomerase II closely correlate with the alterations in the nuclease cleavage pattern around scs and scs' noted in previous chromatin structure studies (31). This would suggest that changes in the nucleoprotein structure of scs and scs' after induction may open up sites in and around these two elements for topoisomerase action. Enzyme moieties interacting with these newly exposed sites could then function to relieve torsional strain generated from within the 87A7 chromatin domain by the heavy transcription of the hsp70 genes.

Recovery. When heat-shocked cells are shifted from 37 back to 25°C, transcription of the *hsp70* genes gradually shuts off and the 87A7 puff regresses. While torsional strain generated by transcription probably drives decondensation, compacting of the locus during recovery is presumably promoted by the reestablishment of protein-DNA and protein-protein interactions that were disrupted by transcription. The recondensation of the 87A7 chromomere during recovery appears to be initiated at the edges of the domain and then spreads inward toward the two genes. We first observe the reestablishment of the preinduced scs and scs' nucleoprotein structures (31) and the concomitant disappearance of the heat shock-specific topoisomerase II cleavage products around each of these putative domain boundaries (summarized in Fig. 8B). This redistribution is completed well before the preinduced topoisomerase II cleavage pattern is restored around the *hsp70* genes. Consequently, it seems likely that topoisomerase II localized at scs and scs' may play only a minor role in the topological transitions which occur within the 87A7 domains as the chromatin fiber recondenses.

The major sites of action for topoisomerase II when the chromatin fiber recondenses during recovery are located in the intergenic spacer and the *hsp70* genes (Fig. 8B). Perhaps the most interesting of these is site 3 (Fig. 8). Although it is the major topoisomerase II site in 87A7 prior to induction, it is not cleaved when the genes are expressed at high levels. During recovery, it is the first of the preinduced products to reappear, and it becomes progressively stronger so that the (relative) yield appears to be even greater than it is prior to induction (Fig. 3). Another site which may play a role in the recondensation process is site 6, which is located near the 5' ends of both *hsp70* genes. This site is unusual in that it is prominent both before and after induction. Moreover, during recovery, cleavage at this site remains high at a time when the yield of products from the heat shock sites in the gene (e.g., sites 7 to 9 [Fig. 3]) has dropped substantially and the yield of products from the preinduction sites (e.g., sites 4 and 5) is still relatively low. These results may indicate that topoisomerase II localized at sites 3 and 6 (and perhaps also at the two weaker sites in the intergenic spacer) may be important in facilitating the topological transitions that occur when the heat shock locus is condensed from a predominantly beads-on-a-string nucleosome array into a coiled 30-nm fiber.

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