Unrestrained DNA supercoiling and the number of topological domains were measured within a 1.8 megabase pair chromosomal region consisting of about 200 tandem repeats of a mouse mammary tumor virus promoter-driven ha-ras gene. When uninduced, unrestrained negative supercoiling was organized into 32-kilobase pair (kb) topological domains. Upon induction, DNA supercoiling throughout the region was completely relaxed. Superc fouling was detected, however, when elongation was blocked before or following induction. The formation of transcription initiation complexes upon addition of dexamethasone decreased the domain size to 16 kb. During transcription the domain size was 9 kb, the length of one repeat. These results suggest that topological domain boundaries can be “functional” in nature, being established by the formation of activated and elongating transcription complexes.

Topological domains, often referred to as chromosomal loops, have been detected in many organisms including Escherichia coli (1), Drosophila (2), and humans (3) (for review see Ref. 4). The formation of a topological domain requires restraint of the DNA helix such that rotation of one strand of the DNA double helix around the other is prevented. Consequently, DNA within a topological domain can contain a linking number deficit that is manifest as unrestrained superhelical energy (for review see Ref. 5). Domain boundaries may result from the attachment of the DNA at specialized sites (i.e. MARs or SARs) (6, 7) onto the nuclear matrix. Recently a bipartite sequence element may have been identified within matrix attachment regions that may be important in chromosome organization or function via these sites (8). Alternatively, topological domain boundaries may be functional in nature resulting from the attachment of functional proteins such as RNA or DNA polymerase complexes to the nuclear membrane (9). For example, in Salmonella typhimurium membrane attachment of TetA leads to the formation of a topological domain boundary defined by the transcriptional complex where the RNA is being translated (10). In addition, in yeast, telomeric sequences can act as functional anchor points for chromosome organization to provide blocks to the transmission of supercoils across the block (11). Other DNA-enzyme complexes in bacteria, such as that created by UvrAB can also act as a boundary to supercoiling (12).

DNA in living bacterial cells is organized with a linking number deficit leading to a state of unrestrained negative supercoiling (1, 13). Although, DNA is negatively supercoiled in bacterial cells, not all supercoiling is unrestrained. About half the supercoils in DNA in Escherichia coli are restrained, possibly by the wrapping of DNA around histone-like proteins (14–18). Consequently, in vivo measurements of levels of supercoiling are about half that measured for the purified chromosome (19–22). Initial studies of supercoiling in eukaryotic cells failed to detect unrestrained supercoiling averaged over the entire chromosome (15), presumably due to the restraint of supercoils through the organization of DNA into nucleosomes. However, analyses of individual genes in Drosophila, mouse, and human cells have revealed the presence of unrestrained supercoiling associated with gene regions (2, 23–26), whereas DNA outside the functional hsp70 domain at locus 87A Drosophila was completely relaxed (2). However, a relationship between transcription or transcriptional activation and unrestrained supercoiling remains to be clearly established. For example, the level of supercoiling within a transcriptionally active hygromycin resistance gene introduced into different regions of the human genome can vary from highly supercoiled to relaxed (26).

The mouse mammary tumor virus promoter provides a model system in which the nucleosomal organization and nucleosomal reorganization upon transcription activation are extremely well characterized (27). Transcription rapidly follows the addition of the glucocorticoid hormone dexamethasone, allowing analysis of the chromatin under conditions of gene repression or activation. When stably introduced into the genome, the mouse mammary tumor virus (MMTV) promoter acquires six positioned nucleosome families (28), positioned over the binding sites for the glucocorticoid receptor and associated factors involved in promoter activation. Steroid hormone induction of the MMTV promoter with dexamethasone renders the DNA sequences associated with the B nucleosome family accessible to restriction endonuclease digestion (28). The
Transcription-induced Topological Domains

28591

hormone-dependent increased nucleosome accessibility is believed to be mechanistically responsible for the loading of transcription factors and the subsequent induction of transcriptional activation (29, 30). The increase in enzyme accessibility of nucleosome B in a stably integrated MMTV promoter is 15–20% in one mouse cell line, suggesting that transcriptional activation of the promoter occurs at similar levels (31).

The well-characterized MMTV promoter and the availability of a cell line containing multiple tandem copies of this promoter afford an excellent system in which to study the effects of gene activation and transcriptional elongation on the level of DNA supercoiling and the number of topological domains. We have studied the change in supercoiling and topological domain size in a DNA region containing approximately 200 copies of the MMTV promoter 5′ of the ha-v-ras gene to understand the role of chromatin organization and supercoiling on gene expression. The transcriptional state of the gene influences the topological domain size and the level of unrestrained supercoiling. The average domain size within the tandem array of MMTV promoter-driven genes changes upon transcriptional activation and elongation suggesting that transcription complexes are functional topological domain boundaries.

EXPERIMENTAL PROCEDURES

Cells, Cell Growth, and DNA Purification—Cell line 3134 contains approximately 200 copies of the 9-kb fragment (pM18D) of the plasmid pM18 (28) in a tandem array in a single chromosomal location. Cells were maintained as a monolayer in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum at 37 °C in a 5% CO2 atmosphere. To incorporate BrdUrd into the DNA, cells were grown in Dulbecco’s modified Eagle’s medium lacking phenol red and containing 10% charcoal-stripped serum (HyClone). The culture medium was changed every 3–4 days. Cells were harvested and treated with glyoxal aldehyde and methylsulfonyl fluoride, 1% Triton X-100. The homogenate was diluted 1:1 with digestion buffer (25% glycerol, 5 mM magnesium acetate, 10 mM HEPES, pH 7.8, 0.5 mM diithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1% Triton X-100). The homogenate was diluted 1:1 with digestion buffer (25% glycerol, 5 mM magnesium acetate, 10 mM HEPES, pH 7.8, 0.5 mM diithiothreitol, 0.1 mM EDTA), and centrifuged through a pad containing digestion buffer for 15 min at 1,000 × g and 4 °C. Nuclear pellets were resuspended in 25% glycerol, 5 mM HEPES, pH 7.8, 0.1 mM EDTA, 0.5 mM diithiothreitol. 10 μg of DNA equivalent per sample was reannealed with 100 units of S1 nuclease at 37 °C in 50 mM NaCl, 50 mM Tris-Cl, pH 8.0, 0.5 mM MgCl2, 1 μM β-mercaptoethanol (35) extracted with phenol-chloroform and precipitated with ethanol. The DNA was cut to completion with DpnII before primer extension to determine the fractional cleavage. To obtain a linear amplification of the signal, primer extensions with Tag polymerase were thermally cycled in 50 mM KCl, 50 mM Tris-Cl, pH 8.8, 200 μM dNTP, 3.5 mM MgCl2, 0.1% Triton X-100, using an oligonucleotide (range of fragments, 8.3 to 48.5 kb). The separated genomic DNA was collected from the bottom of the tube onto 3-cm square sections of a nylon membrane (31) by capillary action under alkali conditions. This blot was hybridized with the 3.0-kb S1 fragment was isolated from plasmid pF-3134 cells, lysed in agarose blocks, was digested overnight at 37 °C in 80% formamide, 0.2 M NaCl, 1 mM EDTA, 40 mM PIPES, pH 6.4, for 6–12 h at 37 °C. Hybrids were washed at 65 °C in 2× SSC at 65 °C for 60 min. Quantitation was completed using a Molecular Dynamics PhosphorImager using ImageQuant software. The fraction of DNA cross-linked (Fx) was calculated by dividing the area of the cross-linked peak by the sum of the area for the cross-linked and noncross-linked peaks. The cross-links per kilobase (Xi/kb) were calculated using the formula Xi/kb = −ln(1 − Fx/S), as described (24), where S is the size of the restriction fragment (S = 3.0 kb for the SpeI fragment of pM18). RUn values represent an average of two Southern blots each from a minimum of three separate experiments. The ratio of the mean cross-linking rate in intact versus relaxed domains (RUn = Xi/kb/Xi/kb,R) reflects the level of unrestrained supercoiling (24). An unpaired, two-tailed, t test was performed with these RUn values, and a significant difference is indicated if p < 0.05.

Measurement of Single Strand Breaks—The frequency of single strand breaks introduced by BrdUrd photolysis was determined as described previously (26) with the following modifications: 5–7 ml of 50 mM Tris base pH 7.0–8.0 (pH range of 7.2–8.2) containing 10 mM dithiothreitol, 0.5% Triton X-100, 0.1% SDS (range of fragments, 8.3 to 48.5 kb). The separated genomic DNA was analyzed by hybridizing to the BPV RNA probe.
fluid and counted for radioactivity. Molecular weights of the single strand DNA fragments (Z) were calculated (41), and the number of single strand breaks per tandem array was calculated using a molecular weight of 338 g/mol bp and $8 \times 10^6$ bp per tandem array.

RESULTS

Characterization of Cell Line 3134—Cell line 904.13 was originally established by transformation of C127 cells with a bovine papilloma virus vector containing the MMTV promoter driving expression of the \textit{ha-v-ras} gene (Fig. 1C). Cell line 3134 was derived from the 904.13 line after a spontaneous event resulting in the integration and amplification of the episome. CHEF gel analysis (Fig. 1A) of DNA from this cell line with three different restriction enzymes, which do not cut the circular DNA construct, resulted in fragments ranging in size from 1.8 to 2.2 \times 10^6 base pairs. Partial digestion with a single-cut enzyme produced a ladder of fragments with a repeat size of 9 kb (Fig. 1B). These data indicate that the cell contains a large tandem array with approximately 200 head to tail copies of the original 9-kb fragment (Fig. 1C). Details of the 9-kb repeat unit are diagrammed in Fig. 1D.

A 3,026-bp \textit{Spe}I fragment encompassing the MMTV promoter was used to assess the level of unrestrained supercoiling within the inserts of the tandem array (Fig. 1D). This fragment contains the entire MMTV LTR, and most of the transcribed region of the \textit{ras} sequence. The percentage of MMTV templates activated by dexamethasone in this cell line was assessed by hypersensitivity to \textit{SacI} cleavage, which cuts in the nucleosome B region of the promoter (Fig. 1D) (29, 31, 42). In a representative experiment, about 7–8% of the \textit{SacI} sites were accessible in control cells or in cells treated with \textit{α}-amanitin (Fig. 2; \textit{Control} and \textit{Amanitin}). Treatment with dexamethasone resulted in an increase in the accessibility of this region, approximately 23% of the promoters were cut (Fig. 2; \textit{Dex}). This represents an increase of 0.15–0.16 in the fractional cleavage, in agreement with previous studies (31, 42) and suggesting that 15–20% of the promoters are active at the time of the assay. In addition, treatment with dexamethasone following a 1-h pretreatment of the cells with \textit{α}-amanitin resulted in a similar increase in fractional cleavage (Fig. 2; \textit{Dex + Amanitin}), indicating that remodeling of the Nuc-B region occurs independently of transcription, as suggested by others (43). \textit{α}-Amanitin inhibits MMTV-driven transcription under our incubation conditions as illustrated by a representative experiment in Fig. 3. Specific transcription, or more properly RNA accumulation, was examined with an S1 protection assay. 3134 cells treated with dexamethasone for 1 h displayed a 7–8-fold increase in RNA detected relative to uninduced cells (Fig. 3; \textit{Control} and \textit{Dex}), comparable with previous determinations in the parental 904.13 cell line (44). Incubation of the cells with 50 μg/ml \textit{α}-amanitin for 1 h before dexamethasone treatment resulted in approximately a 1.5–1.7-fold increase in transcript levels relative to control cells (Fig. 3; \textit{Amanitin} + \textit{Dex}). This represents a substantial 90–95% decrease in the steady state RNA level induced by dexamethasone after 1 h. A 50–70%
MMTV-driven RNA synthesis. Total RNA was extracted from treated or untreated 3134 cells, and the extracted DNA cut to completion with DpnII. Analysis of the cleavage by primer extension was conducted with an oligonucleotide priming from +27 in Nucleosome A toward the Nucleosome B region. The chart shows the fractional cleavage by SacI in cells treated as follows: untreated (control); α-amanitin for 1 h (amanitin); dexamethasone for 1 h (Dex); α-amanitin for 1 h followed by dexamethasone and additional incubation for 1 h (Dex + amanitin). Results are for a representative experiment. Results from duplicate experiments were similar.

![Fig. 2. Steroid-dependent hypersensitivity of the MMTV promoter is refractory to α-amanitin treatment.](image)

FIG. 2. Steroid-dependent hypersensitivity of the MMTV promoter is refractory to α-amanitin treatment. Nuclei were prepared from treated or untreated 3134 cells, digested with SacI, and the extracted DNA cut to completion with DpnII. Analysis of the cleavage by primer extension was conducted with an oligonucleotide priming from +27 in Nucleosome A toward the Nucleosome B region. The chart shows the fractional cleavage by SacI in cells treated as follows: untreated (control); α-amanitin for 1 h (amanitin); dexamethasone for 1 h (Dex); α-amanitin for 1 h followed by dexamethasone and additional incubation for 1 h (Dex + amanitin). Results are for a representative experiment. Results from duplicate experiments were similar.

decrease in the basal transcription of uninduced templates was also observed in cells treated with α-amanitin without dexamethasone (Fig. 3; Amanitin); however, the quantitation in this case is not as accurate because of the low RNA levels.

Psoralen Accessibility and Unrestrained Superciling at the MMTV Promoter—The rate of Me2-psoralen cross-linking to DNA depends on two parameters: the accessibility of the DNA and the level of unrestrained supercoiling in the DNA. Accessibility is dependent upon the extent of association of DNA with nucleosomes or other proteins that prevent Me2-psoralen binding (37). Psoralen accessibility of the MMTV promoter in this mouse cell was analyzed in four sets of cells: untreated (control) cells where the ha-u-ras gene was inactive; dexamethasone-treated cells that were transcriptionally active; cells treated with dexamethasone followed by α-amanitin, in which transcription elongation was allowed to proceed and then was blocked; and cells treated with α-amanitin followed by dexamethasone, in which the promoter was activated but transcription elongation was prevented. Each set of cells was treated with psoralen and exposed to increasing doses of 360-nm light. The DNA was irreversibly denatured as described under “Experimental Procedures,” and the cross-linked and noncross-linked molecules were separated on a native agarose gel (Fig. 4A). The cross-links per kilobase (Xl/kb), calculated from the intensities of the cross-linked (Xl) and noncross-linked (non-Xl) SpeI fragments (Fig. 4A), showed a linear relationship as a function of light exposure for each set of cells (Fig. 4B). As shown in Fig. 4B, the cross-linking rates for the cell lines were linear to at least 12 kJ/m2. The molecular bases for the slight decrease in rate of binding in cells treated with dexamethasone (permeability or accessibility changes) was not investigated. In the experiments to determine the level of unrestrained negative superhelical energy, a dose of 6 kJ/m2 of 360-nm light was chosen because it is within the linear range of the cross-linking reaction.

Unrestrained supercoiling was determined by comparing the XI/kb at a constant dose of psoralen and light within the SpeI fragment before and after the chromosomal DNA was nicked by BrdUrd photolysis. In Fig. 5A the intensity of both the XI and non-XI species decreased at higher doses of nicking but proportionally the XI band decreases faster (determined by PhosphorImager analysis). Thus, the rate of cross-linking (or XI/kb ver-

![Fig. 3. Incubation of 3134 cells with α-amanitin inhibits MMTV-driven RNA synthesis.](image)

FIG. 3. Incubation of 3134 cells with α-amanitin inhibits MMTV-driven RNA synthesis. Total RNA was extracted from treated or untreated 3134 cells, and MMTV LTR reporter-specific RNA assayed by S1 hybrid protection. The single-stranded probe spanned treated or untreated 3134 cells, and MMTV LTR reporter-specific RNA was assayed by S1 hybrid protection. The single-stranded probe spanned treated or untreated 3134 cells, and MMTV LTR reporter-specific RNA was assayed by S1 hybrid protection.

![Fig. 4. Rate of psoralen cross-linking within the 1.8-megabase pair array.](image)

FIG. 4. Rate of psoralen cross-linking within the 1.8-megabase pair array. A, Southern analysis on DNA from dexamethasone-treated cells cross-linked for various times after exposure to psoralen. B, a plot of the XI/kb as a function of light dose. Cells were treated with 360-nm light at an incident intensity of 1.2 kJ/m2/min. Samples were removed after various times and analyzed by Southern hybridization to determine the XI/kb as described under “Experimental Procedures.” ○, untreated; □, dexamethasone followed by α-amanitin treatment; △, dexamethasone; 224 , α-amanitin followed by dexamethasone treatment. Lines represent the linear regression, best fit to the data. Lines for the dexamethasone and the α-amanitin followed by dexamethasone treatment data are identical.
Measurements of Topological Domains in 3134 Cells—Different times of irradiation with 313-nm light that introduced nicks into the DNA were required to relax all supercoils in cells following the different treatments shown in Fig. 5B. In untreated cells, the relaxation of most supercoiling (>95%) within the SpeI fragment occurred after 5 min of nicking (Fig. 5B, Control), but relaxation required greater than 15–18 min of nicking in cells treated with dexamethasone and then α-amanitin or in cells treated with α-amanitin and then dexamethasone (Fig. 5B, Dex + α-amanitin, α-amanitin + Dex). The different doses of 313-nm light required for nicking suggest that the domain size in the tandem array may be different in the nontreated and treated mouse cells.

The psoralen assay for measurement of a topological domain size requires that the DNA contain unrestrained negative supercoils, and the assay measures the loss of superhelical tension as a function of the number of nicks introduced into DNA (37–39). The number of nicks introduced into the DNA by photolysis of BrdUrd-labeled DNA was measured by alkaline sucrose gradient sedimentation analysis. Alkaline sucrose gradient fractions were collected onto a nylon membrane and probed using Southern hybridization to determine the average molecular weight of the DNA containing the MMTV inserts within the tandem array. From this analysis, the number of strand breaks introduced into the MMTV tandem array as a function of the 313-nm light dose could be calculated.

The nicking rates were not significantly different statistically between the nontreated cells and cells treated with α-amanitin, dexamethasone, or any combination of the two. However a statistically significant 2-fold difference was observed between the cells that were treated with dexamethasone then α-amanitin and the cells that were treated with α-amanitin then dexamethasone (Fig. 6, insert, top and bottom lines). Treatment with dexamethasone followed by α-amanitin yielded the highest number of strand breaks, and this treatment may have resulted in the most open chromatin configuration. Treatment with α-amanitin would be expected to keep the chromosome more organized into nucleosomes and thus more protected from free radical attack during BrdUrd photolysis.

To estimate the number of topological domains, it is assumed that the nicks are introduced in a Poisson distribution (1, 38, 39). Using the Poisson formula, theoretical curves are calculated to determine the best fit with the experimental data points (described in the legend to Fig. 6). In Fig. 6, three sets of experimental data are plotted against five theoretical curves each representing a different topological domain size. The theoretical curve that best fits the experimental data represents the domain size. The average number of topological domains/tandem array for untreated cells was 55/tandem array, or 32,000 bp/domain. For cells treated with α-amanitin and then dexamethasone, the number of domains was approximately 110/tandem array, or 16,000 bp/domain. For cells treated with dexamethasone and then α-amanitin approximately 215 domains/tandem array were present, with 8,000–9,000 bp/domain.

Treatment with dexamethasone or α-amanitin should not introduce major changes in chromosome structure that would influence the results obtained. The effects of dexamethasone
have been extensively studied in the MMTV promoter (28, 45) and the TAT promoter (46). In both of these genes, dexamethasone is known to have very local effects on chromatin structure. Therefore, treatment with dexamethasone would not be expected to have global chromatin effects. In addition, no significant effect of dexamethasone treatment on the rate of nicking was observed when looking at the DNA size averaged over the entire chromosome (data not shown). In a previous analysis of a hyg gene in five random chromosomal locations in human cells, no unexpected differences in the rate of cross-linking were observed following α-amanitin treatment, other than those expected for a loss of supercoiling (26). Moreover, in two cell lines, no supercoiling was observed, and α-amanitin had no effect of cross-linking rates. In Drosophila experiments, using heat shock to induce the hsp70 genes, very little change was observed in the rRNA gene response to nicking or heat shock. In addition, differential changes were observed throughout loci 87A7, which were specific for the hsp70 transcription units, the flanking nontranscribed regions, and scs and ses’ sequences (2). These total experiments argue against any global genome wide effects of induction of specific genes or α-amanitin treatment that would complicate the interpretation of the results.

**DISCUSSION**

In this study the effect of transcriptional activation/elongation on unrestrained supercoiling and its relation to topological domain size was examined. Studies were performed on the MMTV promoter-driven ha-v-ras gene inserted in the chromosome of a mouse cell line. Analysis was completed using a psoralen-based assay for unrestrained negative supercoiling in combination with measuring the rate of loss of supercoils following the introduction of nicks into DNA by BrdUrd photolysis. Our results demonstrate that this MMTV promoter-driven ha-v-ras gene is maintained with a moderate level of unrestrained torsional tension in the uninduced state. Transcription elongation decreased negative supercoiling. This is the first example of a gene that becomes torsionally relaxed upon transcription. Transcriptional activation had little or no effect on supercoiling, and blocked elongating complexes also had little or no effect on negative unrestrained supercoiling. However, the activation of transcriptional complexes and the presence of elongating transcriptional complexes increased the number of topological domains within the MMTV promoter-gene region.

Unrestrained negative supercoiling in the tandem ha-v-ras genes was relaxed following the activation of transcription by addition of dexamethasone. This result is in contrast with certain models in which supercoiling has been thought to be a prerequisite for transcription and possibly a consequence of RNA polymerase movement during elongation (for example, see Ref. 4). In fact, transcription in the Drosophila hsp70 locus results in a slight increase in an already high level of unrestrained supercoiling (24). In the case of the ha-v-ras gene, the release of unrestrained supercoiling during active transcription may be due to the active recruitment or activation of topoisomerases within the locus.

Unrestrained supercoiling was maintained in cells treated with any combination of dexamethasone and α-amanitin; i.e. cells induced for transcription but in which active transcriptional elongation was inhibited. The level of unrestrained supercoiling in these cells (RI/N = 1.43, 1.48) was similar to that in uninduced cells (RI/N = 1.55). The slight decrease in supercoiling in cells treated with α-amanitin may be due to residual active transcription (90–95% of transcriptional elongation has been blocked, Fig. 3). Supercoiling was reestablished in cells treated with dexamethasone and then α-amanitin upon the inhibition of transcription elongation. The reestablishment of supercoiling occurred within the 1 h between α-amanitin treatment and the psoralen photobinding. If active transcriptional elongation is tightly coupled with the active topoisomerase(s) it is understandable that negative supercoiling could be rapidly relaxed. It is known that topoisomerase I is associated with regions of active transcription (47–49), and that it is also recruited to promoters by transcription factor IID (50, 51). That the inhibition of elongation leads to reestablishment of unrestrained supercoiling implies a mechanism exists for the regeneration, or loss of restraint, of supercoils. This mechanism does not appear to be dependent upon tracking by RNA polymerase. Furthermore, current evidence indicates that no loss of nucleosomes occurs from the LTR during activation (31, 52).

**Changes in Topological Domain Organization Occur During Transcription of the ha-v-ras Gene—**The topological domain size changed dramatically upon transcription. In the mouse cell line 3134 there are approximately 200 tandem inserts containing the MMTV promoter, each insert is 9,000 bp in length, and the entire region consists of 1.8 × 10⁶ bp. In cells in which the ha-v-ras gene was inactive approximately 55 topological domains existed, such that each domain was, on average, 32,000 bp in length. This is similar to the size found in the rDNA genes in humans fibrosarcoma cells (26). These domain boundaries may be defined by some structural attachment to a nuclear structure. Cells treated with α-amanitin and then dexametha-
A, loading a large initiation complex, including RNA polymerase II, on the promoter provides a constraint to DNA rotation that effectively creates a new topological domain. B, receptor activation recruits the active promoter units to a nuclear structure that serves as an active center for transcription. These centers represent the domain boundaries.

The observations that transcription can influence a topological domain size suggests several mechanisms for domain organization. In Fig. 7, top panel, the polymerase complex could establish a topological domain by simply preventing rotation of the DNA double helix. Polymerase complexes bound to the promoter sequence and polymerase complexes transcribing the gene can establish a topological domain. In this case rotation is prevented by restraints to rotational diffusion. Alternatively, in Fig. 7, bottom panel, the polymerase complex is recruited to a nuclear structure upon gene activation. Alternatively, the transcriptional machinery is localized at a nuclear structure and activation involves the association of the DNA with the localized, transcription complexes. In this case the DNA template feeds through the attached complex during elongation, as suggested for the tetA gene in E. coli (53). Attachment to a nuclear structure would clearly prevent rotation of the DNA and establish a topological domain.

In conclusion, our results establish that unrestrained supercoiling is present in the tandem array of MMTV inserts within a mouse cell. Active transcription decreases the level of unrestrained supercoiling within this gene. Remarkably, the topological domain size decreases when the MMTV promoter is activated by dexamethasone, and a further reduction is observed if an actively transcribed ha-ras gene is inhibited by α-amanitin. These results demonstrate that the transcriptional state of a gene can influence both the level of unrestrained supercoiling and the topological domain size. Although certain topological domains may be defined by the interaction of MARs with a nuclear structure, clearly some topological domains in eukaryotic cells appear to be defined, in some way, by active transcription complexes.

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