Transcription and DNA supercoiling

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Transcription and supercoiling of the DNA template are interrelated. This review summarizes recent progress in the study of how template topology affects transcription, and how transcription affects template topology inside wild-type and DNA topoisomerase mutant cells. The interplay between DNA supercoiling and transcription raises interesting questions on the regulation of adjacent genes, the organization of intracellular DNA, and the coupling between transcription and other cellular processes involving DNA.

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Introduction

It is well known that the supercoiling of DNA may affect its secondary structure and its interactions with other molecules. In Figure 1, a DNA segment within a hypothetical protein–DNA complex is shown to possess a loop with a negative writhe as well as an unwound region. These loops are more easily formed in negatively supercoiled DNA than in relaxed DNA, therefore negative supercoiling of a relaxed DNA is expected to stabilize such a protein–DNA complex, whereas positive supercoiling is expected to destabilize it.

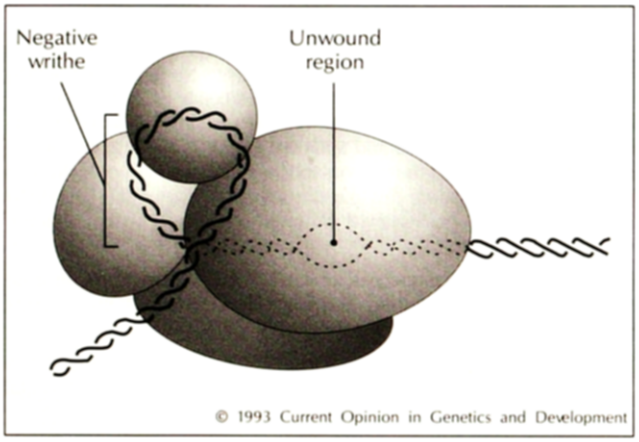

Fig. 1. A hypothetical protein–DNA complex in which the bound DNA segment possesses a loop with a negative writhe and an unwound region.

An 'open' transcription complex with a short stretch of unpaired DNA is often found to form more readily in a negatively supercoiled DNA than in a linear or relaxed DNA. However, in transcription complexes containing a multitude of catalytic and regulatory proteins, it is difficult to assess the sum of the changes in the twist and writhe of the bound DNA segment brought about by the various protein components. Thus, in general, experimental measurements are required to determine how template supercoiling affects the transcription of a particular gene. This is also true in cases where the efficiency of RNA synthesis is determined by the kinetics of formation rather than the thermodynamic stability of a particular complex.

In recent years, evidence has been accumulating to show that the transcription process itself may also affect supercoiling of the template. Figure 2 illustrates the 'twin-domain' model of transcriptional supercoiling (R represents the transcription machinery and E a cellular entity associated with the DNA ring; E can also be viewed as cellular entities associated with the boundaries of a chromosomal DNA loop within which R is located) [1]. The model predicts that the steady state levels of supercoiling of the domains in a DNA ring or loop are dependent on the rate of transcription, the magnitudes of the forces that retard the rotation of R and E, and the actions of the DNA topoisomerases.

In this article, we summarize recent advances on how DNA supercoiling and transcription are interrelated. Earlier references can be found in recent reviews [2,3] and in the literature citations of the listed references.

Effects of template supercoiling on eukaryotic transcription systems

Studies of rRNA synthesis by RNA polymerase I (pol I) in extracts of yeast cells free of DNA topoisomerase activity indicate that chain initiation is more efficient on negatively supercoiled DNA than on linear DNA [4]. The authors also found that for pol I promoter-driven chromosomal genes several kilobase pairs in length, transcription in yeast is reduced upon inactivation of both DNA topoisomerases I and II, which they attribute

Abbreviations

MLP—major late promoter; ***pol***—polymerase; $-\sigma$—superhelical density.

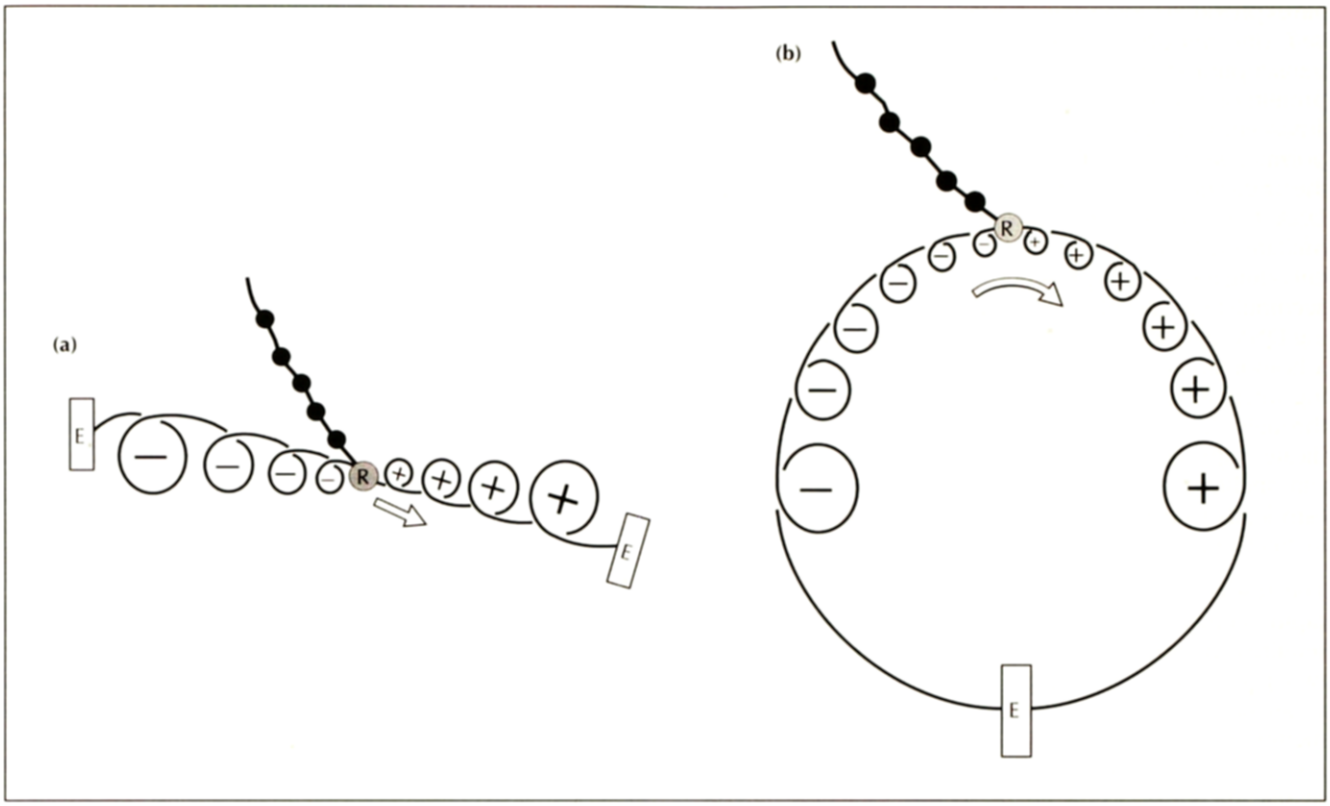

Fig. 2. The twin-domain model of transcriptional supercoiling. **(a)** A transcriptional ensemble R, including the RNA polymerase, the nascent RNA and RNA-associated proteins, is shown tracking along its DNA template (in the direction indicated by the arrow); the bars denoted by E represent hypothetical cellular entities that are involved in the organization of intracellular DNA into loops. When the rotation of R and E around the DNA is retarded or prevented, positive supercoils (+ signs) would be generated ahead of the translocating R, and negative supercoils (− signs) behind it. **(b)** The two bars denoted by E in **(a)** can be combined to illustrate the supercoiling of an intracellular DNA ring by transcription. (For further details of the model, see [1,2,9•]).

to hindrance of chain elongation when positive supercoils accumulate downstream of the nascent transcripts in the absence of the topoisomerases. These findings are in accordance with earlier results on rRNA synthesis in yeast and in mammalian cell lines.

The availability of purified components of eukaryotic pol II-dependent transcription systems has led to the finding that in the presence of the basal factors TBP, TFIIA/J, TFIIB, TFIIE, TFIIF, and TFIIH, transcription of either the immunoglobulin heavy chain (IgH) promoter or the adenovirus major late promoters (MLPs) by pol II is more efficient with a negatively supercoiled DNA template than a linear one. In general, the higher the negative specific linking difference or superhelical density ($-\sigma$) of the DNA template, the higher the level of RNA synthesis; a slight reduction in RNA synthesis has been observed, however, when $-\sigma$ exceeds 0.07 [5•]. Interestingly, pol II plus TBP and TFIIB can accurately and efficiently transcribe from an immunoglobulin heavy chain promoter on a negatively supercoiled template, but not from the same promoter on a linear template; pol II transcription from the MLPs on either negatively supercoiled or linear template, however, requires the presence of the other basal transcription factors as well.

The observed increase in the level of RNA synthesis with $-\sigma$ until a high value of $-\sigma$ is reached, and the ability of a 'core enzyme' to transcribe negatively supercoiled but not relaxed templates are reminiscent of results obtained with bacterial RNA polymerases. A key determinant in these phenomena appears to be the facilitation of open-complex formation by negative supercoiling of the template. Different transcription systems appear to differ in how readily an unwound bubble can form within a promoter. At least for some of the eukaryotic pol II promoters, it has been suggested that open complex formation may involve their unwinding by a helicase subunit of TFIIH, the ERCC-3 gene product, that has also been shown to participate in the excision repair of DNA [6•]. The observation that negative supercoiling of DNA favors the initiation of transcription by pol II [5•] is also consistent with the demonstration that mRNA synthesis in yeast is much diminished when intracellular chromosomal or plasmid DNA becomes positively supercoiled [7].

Barriers to rotation of intracellular DNA around its helical axis

As mentioned, the transcriptional supercoiling of intracellular DNA is predicted to depend on barriers that prevent or retard the transcriptional machinery from circling around the DNA or the DNA from rotating around its longitudinal axis. Two recent papers

have provided additional evidence that in *Escherichia coli*, one important class of barriers involves coupled transcription–translation of a gene encoding a membrane protein or a protein for export; membrane association of a nascent polypeptide may anchor the transcribing RNA polymerase through the mRNA tether, thus preventing the transcriptional machinery from circling around the DNA. Cook *et al.* [8•] showed that when a plasmid simultaneously expresses two oppositely oriented genes encoding membrane proteins, negative supercoils rapidly accumulate in the plasmid in an *E. coli topA* strain lacking DNA topoisomerase I. Lynch and Wang [9•] analyzed a homologous set of plasmids expressing genes encoding integral membrane proteins or proteins for export through the cytoplasmic membrane. Their results indicate that cotranscriptional synthesis of such proteins can anchor the transcribing RNA polymerase, which in turn leads to hypernegative supercoiling of the template in *topA* strains.

The results of Cook *et al.* [8•] demonstrate nicely that rapid accumulation of negative supercoils in a plasmid requires the simultaneous anchoring of a minimum of two points. If only a single point on a circular DNA is anchored through cotranscriptional synthesis of a membrane-bound polypeptide or by any other mechanism, oppositely supercoiled domains on the two sides of the anchoring point are expected to merge rapidly through rotation of the DNA connecting the domains. In *E. coli*, recent experiments using an inducible site-specific recombinase to form non-replicating DNA rings expressing a single membrane protein gene indicate that such rings do not become hypernegatively supercoiled in a *topA* genetic background (AS Lynch and JC Wang, unpublished data). This approach should be generally applicable in the identification of elements that serve as barriers to rotation of intracellular DNA around its helical axis. In yeast, a similar approach indicates that the *REP1* and *REP2* gene products of the 2μ plasmid can act as such a barrier for DNA containing the *REP3* sequence [10].

An interesting barrier to the cancellation of oppositely supercoiled domains is DNA loop formation through the association of proteins bound to separate points on the same DNA ([11]; for a recent review on loop formation, see [12]). If point A on a plasmid is anchored through cotranscriptional membrane association and points B and C are joined by proteins associated with these sites, a negative supercoiled domain between A and B and a positively supercoiled domain between A and C can not neutralize each other by rotating the connecting BC segment around its helical axis.

Transcriptional supercoiling in cells expressing normal levels of DNA topoisomerases

Whereas the simultaneous generation of positive supercoils ahead of a transcription ensemble and negative supercoils behind it is more readily demonstrated in DNA topoisomerase mutants, several recent experiments have also provided evidence to support the occurrence of transcriptional supercoiling in cells expressing normal levels of DNA topoisomerases. Chemical probing of the supercoiling-sensitive DNA B-to-Z structural transition indicates that in *E. coli*, the region upstream of a promoter experiences an increase in negative supercoiling whereas the region downstream from the terminator experiences the opposite [13]. In addition, transient formation of $d(A\text{-}T)_n$ cruciforms upstream of inducible promoters in *E. coli* cells has been shown to be dependent on transcription [14]. Studies of lac repressor-mediated formation of a DNA loop in *E. coli* have also led to the suggestion that the DNA segment between two divergent promoters is probably highly negatively supercoiled [15•].

In permeablized human U937 cells encased in agarose microbeads, Z-DNA forming segments were found near the c-*myc* gene promoter in cells expressing the gene but not in cells with much reduced c-*myc* expression [16]. Quantitation of psoralen–DNA photoadduct formation in human 6A3 cells before and after X-ray irradiation, which breaks intracellular DNA, has also led to the suggestion that unconstrained negative supercoils might be present in regions near the 5′ ends of the dihydrofolate reductase gene and 45S rRNA transcription unit, and a low level of unconstrained positive supercoils might be present near the 3′ end of the dihydrofolate reductase gene [17].

In eukaryotic cells at least, because the known DNA topoisomerases relax DNA rather than supercoil it, the persistence of unconstrained supercoils in specific domains raises questions concerning the accessibility of various domains to these enzymes. *In vitro* studies of the effect of transcription on γδ resolvase-catalyzed site-specific recombination, which normally requires negatively supercoiled DNA substrates [18•], have also posed a related puzzle; transcription was found to stimulate the recombination, presumably through template supercoiling, yet the presence of a large amount of eukaryotic DNA topoisomerase I showed little effect on the observed dependence on transcription [19•].

Supercoiling of intracellular DNA by mechanisms other than transcription

As pointed out in [1], oppositely supercoiled domains may be generated when a macromolecular assembly translocates along DNA, and supercoiling of the DNA template by transcription is an important, but probably not the only, cause of supercoiling by a tracking mechanism. Several interesting examples have been revealed by recent studies. Confalonieri *et al.* [20•] reported that an ATP-dependent DNA positive supercoiling enzyme found in thermophilic bacteria has a carboxy-terminal domain homologous to eubacterial DNA topoisomerase I and an amino-terminal domain containing several DNA helicase motifs. This finding suggests that the positive supercoiling action of the enzyme is due to the generation of two op-

positely supercoiled domains by its helicase half and the removal of the negative supercoils by its topoisomerase half. Koo *et al.* [21] found that in extracts of yeast cells lacking DNA topoisomerase I, positive supercoils would accumulate in DNA rings in the presence of ATP or dATP and eubacterial DNA topoisomerase I, which specifically removes negative supercoils. These findings, together with the earlier observation that translocation of helicases such as simian virus 40 T-antigen and *E. coli* UvrAB complex along DNA can generate oppositely supercoiled domains, demonstrate that DNA supercoiling by tracking processes is probably fairly common. It is also possible that DNA supercoiling by mechanisms similar to that of eubacterial DNA gyrase may occur in eukaryotes and archaebacteria as well [22]. Two recent studies also suggest the possibility of unconstrained DNA supercoiling in eukaryotes generated by mechanisms other than RNA synthesis [23•,24•].

The interplay between DNA supercoiling and transcription

Because template supercoiling and transcription may affect each other, the possibility arises that the transcription of one gene may activate or deactivate an adjacent gene through a local change in template topology [1], and several recent studies suggest that this might occur. A *Salmonella typhimurium* mutant promoter *leu-500*, for example, is normally inactive but can be activated in a *topA* strain if the promoter is chromosomally located or is placed divergent to a plasmid-borne promoter expressing the TetA membrane protein [25•]. In the latter case, cotranscriptional anchoring of the nascent TetA polypeptide is presumably important for the hypernegative supercoiling of the upstream region, within which the mutant *leu-500* promoter is located. A plausible effect of template topology on gene expression was also reported by Parsot and Mekanalanos [26], who observed that in *Vibrio cholerae*, the normal environmental regulation of a pair of divergently transcribed genes *acfA* and *acfD* was abolished if they were plasmid-borne rather than chromosomally located. A third example in this regard is the transcription of a linear DNA injected into *Xenopus* oocytes; transcription by phage T7 RNA polymerase in the opposite direction from an adjacent promoter can activate the eukaryotic promoter, which is otherwise silent on a linear template [27].

Conclusions

There is now substantial evidence that DNA supercoiling and transcription may affect each other. The twin supercoiled domain model of transcriptional supercoiling also underscores the importance of understanding the relation between supercoiling and the organization of intracellular DNA; what are the barriers that separate supercoiled domains, what kinds of forces might hinder the spiral motion of a transcriptional machinery around its template, and what determines the accessibility of various supercoiled domains to the DNA topoisomerases? The interplay between DNA supercoiling and transcription also illustrates the coupling between various cellular processes. Because DNA supercoiling may have strong effects on its structure and interactions with other cellular entities, the coupling between transcription and supercoiling can be expanded to include potential interplays between transcription and replication, recombination, and other cellular transactions, although these interesting possibilities are outside the scope of this review.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. LIU LF, WANG JC: **Supercoiling of the DNA Template During Transcription.** *Proc Natl Acad Sci USA* 1987, 84:7024–7027.

2. WANG JC: **Template Topology and Transcription.** In *Transcriptional Regulation.* Edited by McKnight S, Yamamoto K. New York: Cold Spring Harbor Laboratory Press; 1992:1253–1269.

3. FREEMAN LA, GARRARD WT: **DNA Supercoiling in Chromatin Structure and Gene Expression:** *Crit Rev Euk Gene Exp* 1992, 2:165–209.

4. SCHULTZ MC, BRILL SJ, JU Q, STERNGLANZ R, REEDER RH: **Topoisomerases and Yeast rRNA Transcription: Negative Supercoiling Stimulates Initiation and Topoisomerase Activity is Required for Elongation.** *Genes Dev* 1992, 6:1332–1341.

5. • PARVIN JD, SHARP PA: **DNA Topology and a Minimal Set of Basal Factors for Transcription by RNA Polymerase II.** *Cell* 1993, 73:533–540.

A demonstration that eukaryotic pol II transcription is no exception to the general rule that template supercoiling may strongly affect open-complex formation.

6. • SCHAEFFER L, ROY R, HUMBERT S, MONCOLLIN V, VERMEULEN W, HOEIJMAKERS JHJ, CHAMBON P, EGLY J-M: **DNA Repair Helicase: a Component of BTF2 (TFIIH) Basic Transcription Factor.** *Science* 1993, 260:58–63.

One of several recent papers that shed light on how transcription and repair might be coupled in eukaryotes and prokaryotes. Also raises again the possibility of open-complex formation by an active process involving one of the pol II associated factors. Whereas all transcription systems require the formation of open-complexes, it seems likely that more than one mechanism that can facilitate DNA unwinding may be involved in the formation of different open complexes.

7. GARTENBERG MR, WANG JC: **Positive Supercoiling of DNA Abolishes mRNA Synthesis in Yeast.** *Proc Natl Acad Sci USA* 1992, 89:11461–11465.

8. • COOK DN, DZWOKAI M, PON NG, HEARST JE: **Dynamics of DNA Supercoiling by Transcription in *Escherichia coli*.** *Proc Natl Acad Sci USA* 1992, 89:10603–10607.

The first examination of the kinetics of transcriptional supercoiling *in vivo*. For a pair of divergently transcribed genes encoding membrane proteins, transcription is shown to efficiently generate positive and negative supercoils in the template, which are efficiently removed at the same time by DNA gyrase and topoisomerase I. The *in vivo* turnover numbers of the topoisomerases can not be estimated from these measurements, however, due to uncertainties in their numbers in each of the oppositely supercoiled domains.

9. • LYNCH AS, WANG JC: **Anchoring of DNA to the Bacterial Cytoplasmic Membrane through Cotranscriptional Synthesis**

of Polypeptides Encoding Membrane Proteins or Proteins for Export: a Mechanism of Plasmid Hypernegative Supercoiling in Mutants Deficient in DNA Topoisomerase I. *J Bacteriol* 1993, 175:1645–1655.

A detailed study of cotranscriptional membrane association of nascent polypeptides and its relation to DNA hypernegative supercoiling when DNA topoisomerase I is inactivated. The results also suggest that cotranscriptional membrane association of proteins for export does not require the presence of signal peptides.

10. GARTENBERG MR, WANG JC: **Identification of Barriers to Rotation of DNA Segments in Yeast from the Topology of DNA Rings Excised by an Inducible Site-Specific Recombinase**. *Proc Natl Acad Sci USA* 1993 (in press).

11. WU H-Y, LIU LF: **DNA Looping Alters Local DNA Conformation during Transcription**. *J Mol Biol* 1991, 219:615–622.

12. MATTHEWS KS: **DNA Looping**. *Microbiol Rev* 1992, 56:123–126.

13. RAHMOUNI AR, WELLS RD: **Direct Evidence for the Effect of Transcription on Local DNA Supercoiling *in Vivo***. *J Mol Biol* 1992, 223:131–144.

14. DAYN A, MALKHOSYAN S, MIRKIN SM: **Transcriptionally Driven Cruciform Formation *in Vivo***. *Nucleic Acids Res* 1992, 20:5991–5997.

15. • LAW SM, BELLOMY GR, SCHLAX PJ, RECORD JR MT: ***In Vivo* Thermodynamic Analysis of Repression With and Without Looping in *lac* Constructs**. *J Mol Biol* 1993, 230:161–173.

This represents a recent illustration of both the possibility of protein-mediated DNA loop formation *in vivo* and the feasibility of deducing the linking deficit of a DNA loop *in vivo* from DNA helical periodicity estimates.

16. WITTIG B, WÖLFL S, DORBIC T, VAHRSON W, RICH A: **Transcription of Human c-*myc* in Permeabilized Nuclei is Associated with Formation of Z-DNA in Three Discrete Regions of the Gene**. *EMBO J* 1992, 11:4653–4663.

17. LJUNGMAN M, HANAWALT PC: **Localized Torsional Tension in the DNA of Human Cells**. *Proc Natl Acad Sci USA* 1992, 89:6055–6059.

18. • KANAAR R, COZZARELLI NR: **Role of Supercoiled DNA Structure in DNA Transactions**. *Curr Opin Struct Biol* 1992, 2:369–379.

An excellent short review on how supercoiling is exploited in various DNA transactions, particularly in site-specific recombinations.

19. • DRÖGE P: **Transcription-Driven Site-Specific DNA Recombination *in Vitro***. *Proc Natl Acad Sci USA* 1993, 90:2759–2763.

The issue of accessibility of DNA topoisomerases to a domain in the neighborhood of a particular point on a DNA is discussed. In this experiment, the supercoiling-dependent formation of a resolvase–DNA complex and the topoisomerase-catalyzed removal of supercoils generated by transcription can be viewed as two competing processes; presumably, the relative initial rates of these processes rather than the steady-state levels of DNA supercoiling are the parameters of importance.

20. • CONFALONIERI F, ELIE C, NADAL M, BOUTHIER DE LA TOUR C, FORTERRE P, DUGUET M: **Reverse Gyrase: a Helicase-like Domain and a Type I Topoisomerase in the Same Polypeptide**. *Proc Natl Acad Sci USA* 1993, 90:4753–4757.

The ability of the 'reverse gyrase' to track along DNA using its helicase activity is yet to be established. The existence of a positive supercoiling activity in thermophilic bacteria and the demonstration that transcription can lead to the generation of positively as well as negatively supercoiled domains in the DNA template, hint at the biological relevance of DNA positive supercoiling.

21. KOO H-S, LAU K, WU H-Y, LIU LF: **Identification of a DNA Supercoiling Activity in *Saccharomyces cerevisiae***. *Nucleic Acids Res* 1992, 20:5067–5072.

22. OHTA T, HIROSE S: **Purification of a DNA Supercoiling Factor from the Posterior Silk Gland of *Bombyx mori***. *Proc Natl Acad Sci USA* 1990, 87:5307–5311.

23. • LEONARD MW, PATIENT RK: **Evidence for Torsional Stress in Transcriptionally Activated Chromatin**. *Mol Cell Biol* 1991, 11:6128–6138.

This paper raised again the interesting issue of the mechanism of activation of chromatin templates for transcription. The inference that this activation is associated with template supercoiling by a process other than transcription, however, is based on the assumption that RNA synthesis is blocked completely by α-amanitin. Whereas it is well-known that α-amanitin blocks pol II elongation, it is less certain whether the inhibitor blocks synthesis of very short transcripts.

24. • JUPE ER, SINDEN RR, CARTWRIGHT IL: **Stably Maintained Microdomain of Localized Unrestrained Supercoiling at a *Drosophila* Heat Shock Gene Locus**. *EMBO J* 1993, 12:1067–1075.

Light-activated DNA crosslinking by psoralen offers a powerful method to assess the binding of a small intercalating agent to bulk intracellular DNA or a particular region of intracellular DNA. Psoralen photobinding is not a simple function of the degree of unconstrained supercoiling, however, which complicates the interpretations. Whether the known synthesis of a short RNA at the hsp70 promoter prior to heat shock might affect template supercoiling is also unknown.

25. • CHEN D, BOWATER R, DORMAN CJ, LILLEY DMJ: **Activity of a Plasmid-borne *leu-500* Promoter Depends on the Transcription and Translation of an Adjacent Gene**. *Proc Natl Acad Sci USA* 1992, 89:8784–8788.

This paper illustrates plausible differences in the local topology of a chromosomally located and plasmid-borne transcription unit.

26. PARSOT C, MEKALANOS JJ: **Structural Analysis of the *acfA* and *acfD* Genes of *Vibrio cholerae*. Effects of DNA Topology and Transcriptional Activators on Expression**. *J Bacteriol* 1992, 174:5211–5218.

27. DUNAWAY M, OSTRANDER EA: **Local Domains of Supercoiling Activate a Eukaryotic Promoter *in Vivo***. *Nature* 1993, 361:746–748.

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