Journal of Cellular Biochemistry 55:93–97 (1994)

Conformational Information in DNA: Its Role in the Interaction With DNA Topoisomerase I and Nucleosomes

Micaela Caserta, Giorgio Camilloni, Sabrina Venditti, Patrizia Venditti, and Ernesto Di Mauro

Centro di Studio per gli Acidi Nucleici, CNR, Rome (M.C., P.V., E.D.M.); Fondazione Istituto Pasteur–Fondazione Cenci Bolognetti, Dipartimento di Genetica e Biologia Molecolare (G.C., S.V., E.D.M.), Università La Sapienza, 00185 Rome, Italy

Abstract Information in DNA is not limited to sequence information. Both local and global conformational parameters are pivotal to the interaction with a number of relevant proteins. The function of the major components of the transcription machinery (RNA polymerase II, DNA topoisomerase I, nucleosomes, the TATA-binding factor) is dependent on the topological status of the substrate DNA molecule. The topological requirements and the conformational consensus that dictate the rules for localization of nucleosomes and define the active sites for DNA topoisomerase I have been established; the reaction of DNA topoisomerase I is regulated by a topological feedback mechanism. The integrating function of the free energy of supercoiling in the transcription process and the regulatory role of DNA topoisomerase I are discussed. © 1994 Wiley-Liss, Inc.

Key words: DNA topoisomerase I, DNA topology, promoter activation, in vitro and in vivo nucleosomes, rotational and translational information

COMPONENTS OF THE TRANSCRIPTION MACHINERY AND THE LOCAL SUPERCOILING

Form of Free Energy

Removal of one individual nucleosome or of a few numerically and topographically well-defined [1–5] nucleosomes from promoters upon induction of transcription has been observed. It is long known that removal of nucleosomes from closed DNA domains releases supercoiling [6], thus making locally available the amount of free energy that corresponds to one superhelical turn. A variation of the free energy in closed domains changes the DNA structure, and this is very likely to have an effect on the interaction with proteins. Thus, removal or displacement of nucleosomes has a structural consequence which becomes immediately genetically relevant. Understanding in detail this phenomenon requires the previous solution of two open problems. The first is how the free energy so made available along the linked (Lk) double strand will be partitioned between variation of the DNA writhing (W) and twisting (T). The general nature of the basic equation of DNA topology—$Lk = T + W$—does not help us in finding a solution to this question. The second problem is whether the surrounding nucleosomes are a valid barrier against the dispersion along the chromatin fiber of the locally available quantum of free energy. Understanding (or at least measuring) this phenomenon is not at close reach.

The helical period of DNA when laid on the nucleosomal surface is defined and is different from the period of DNA in solution. It is therefore unlikely that a local variation of twisting will easily propagate through surrounding nucleosomes without causing general destabilization. On the other hand, dispersion of writhing in the form of torsion along the chromatin fiber is easy to imagine but difficult to evaluate.

Even though answers to these wide-ranging topological questions are lacking, the fact that the removal of one nucleosome from a chromatin string releases free energy may lead to predictions that can be brought to experimental analysis.

These predictions may be formulated in the form of a single general question: how many and

Received November 19, 1993; accepted November 23, 1993.

Address reprint requests to Ernesto Di Mauro, Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza," P.le Aldo Moro, 5, 00185 Roma, Italy.

© 1994 Wiley-Liss, Inc.

which proteins or protein systems use this free energy? And a subquestion: do these proteins compete among themselves for the various structural aspects under which energy is stored?

DNA Topology and Proteins

Among the enzymes and the structural proteins which have been reported to be supercoiling-dependent are RNA polymerases, nucleosomes, eukaryotic DNA topoisomerase I and II, and at least one transcription factor [reviewed in 7]. Limiting our analysis to eukaryotic systems, it is clear that the study of transcription cannot be approached in its entirety without taking into consideration the effect of the variations of supercoiling on the DNA sequences which react with the transcription machinery. We should not forget that if it is true that supercoiling affects transcription, it is also true that transcription affects supercoiling, as described by the twin-domains model of transcription [8]. We shall limit ourselves to two defined pieces of the transcription machinery: nucleosomes and DNA topoisomerase I. We will briefly extend the conclusions to the third major component of the system, RNA polymerase.

At this point, one should recall the following facts about closed DNA systems.

Topology describes the properties that do not change when the (closed) system is modified: the linking number of a closed double strand is a typical topological property. This classical definition is of little practical use. In genetic systems topological properties characterize the closed circles of viral genomes, of plasmids, of intact mitochondrial DNAs, and of in vitro ligated DNAs. In large chromosomal molecules, a chromosomal loop can be considered a topologically defined system. When a nucleosome comes off a string of similar particles, the topological analysis on the 150–200 base-pairs long DNA tract which is left free by its removal is useful, even if it is not formally correct. The description of variations of writhing, of the other topological properties, and of the local variations of the DNA twist is structurally and genetically informative.

For a long time, the issue of unconstrained DNA supercoiling in eukaryotic chromosomes has remained controversial because of the association of DNA with nucleosomes [7]. Recently, the existence of a stably maintained microdomain of localized unconstrained DNA supercoiling has been reported in a *Drosophila* heat shock gene locus [32].

Nucleosomes. Nucleosomes wrap DNA around themselves 1.8 times in a left-handed solenoid. The fact that local flexibility favors the formation of the DNA/protein complex has long been recognized as the driving force of nucleosome formation.

The DNA sequences which allow preferential formation of nucleosomes are characterized by anisotropic flexibility.

The actual sequences which tend to locate themselves inwards relative to the nucleosomal surface and the phase of their distributions have been defined in a series of pioneering studies [9,10 and references therein]. The fact that nucleosomes bend DNA and the phased distribution of flexible sequences predict that intrinsically curved DNA will favor nucleosome formation relative to bulk DNA sequences. The left-handed direction of the curvature on natural nucleosomes predicts that the linkage reduction of DNA will also be a favoring factor. Both predictions have been verified [11–13]. In particular, we have observed that on curved DNAs (from the *Crithidia fasciculata* kinetoplast [12] and in the *Saccharomyces cerevisiae* 5S rRNA repeat gene [13]) multiple nucleosomes are formed in vivo and in vitro which occupy alternative positions constantly on the same rotational phase. This implies that in these systems nucleosomes sit on the same "face" of DNA and enjoy a facilitated sliding possibility. The multiple alternative occupancies spaced by a single helical period are in fact necessarily quasi-isoenergetic, and the possibility of sliding motions has been proposed and verified [14].

In conclusion, the rotational information appears to be the major determinant of nucleosome positioning, to the point that the so far elusive translational information may be nothing but the equilibrium between the two sets of phased flexible sequences distributed at the two sides of the nucleosomal dyad. Of the three terms of the equation of topology (Lk, linking; T, twist; W, writhing) W seems to be the most important in the interaction of nucleosomes with DNA.

DNA topoisomerase I. DNA topoisomerase I changes the linking number of DNA with a nicking-closing mechanism that causes topological relaxation of DNA and does so in a topology-dependent fashion: supercoiled DNA reacts and is relaxed, while unstrained DNA behaves as a

much weaker substrate [15,16]. The fact that the reactivity of the substrate increases as a direct function of its superhelical density (that is, of its writhings) has suggested that DNA curvature plays a role in the interaction with the enzyme. It was actually shown that bent DNA is a highly preferential substrate [17] and the sites which induce curvature are the sites of preferential reactivity, as measured by localization of cleavages [17]. A consensus motif based on conformational parameters, not directly on base sequence, was identified as characteristic of the preferential interaction sites. This motif was described in terms of the set of Eulerian angular values that define the axial path of DNA (helical twist, deflection angle, direction) and of the orthogonal components of wedge (roll and tilt) [18]. In curved DNAs the cleaved sites always map on the external side of the curve [19], and modifications of the tridimensional trajectory of DNA change the reactivity pattern [19].

In conclusion, the reaction of DNA topoisomerase I is highly sensitive to the tridimensional context of its substrate sites, and the reaction is strictly supercoiling-dependent. In the basic equation mentioned above, DNA topoisomerase I is sensitive to each of the three terms: the cleaved sites are characterized by local helical parameters (T), the overall reactivity senses the curvature of the DNA molecule (W), and its reaction changes the linking (Lk) and consequently changes both T and W, bringing the DNA from an active to an inactive state. These studies show that the regulation of the DNA topoisomerase I reaction is a typical feedback regulation, although of a novel type: a topological feedback.

RNA polymerase II. RNA polymerase II enters the DNA at initiation of transcription. In so doing, it changes the local twist and, in closed systems, redistributes twists and writhes. In vitro transcription by purified RNA polymerase II is highly supercoiling dependent, and ternary transcription complexes form on promoters only on negatively stressed systems [20 and references therein]. When contained in topologically closed DNA domains, RNA polymerase II promoters change the local conformation of functionally relevant sites, such as the UAS, the TATA sequences, and the RNA initiation sites, in a coordinate and topology-related way [21]. Evidence for the transmission in cis of topological effects and for the correlation of these effects with activation of transcription in vitro has been obtained [21 and references therein]. Thus, activation of the function of RNA polymerase II changes both the T and the W term of the basic equation of DNA topology. Only the Lk term, the linking number of the molecule, is left intact.

Therefore, coming back to the original question, concerning the genetic consequences of the free energy which is made available on a promoter region upon removal of a nucleosome, it is clear that these consequences are profound. Deposition of nucleosomes is favored by linkage reduction and by alteration of twist, RNA polymerase II is activated, and DNA topoisomerase I starts its nicking-closing reaction. These activities are correlated, are in reciprocal competition, and are largely conflicting; each protein tends to use for its own binding and reaction the locally available free energy. The major role in this process is played by DNA topoisomerase I, due to the fact that its relaxing activity completely and covalently removes the topological strain. The next section considers the hypotheses and the emerging evidences on the involvement of DNA topoisomerase I in eukaryotic transcription.

DNA TOPOISOMERASES AND EUKARYOTIC GENE EXPRESSION: CONTROL OF DNA STRUCTURE AND FUNCTION

In the last decade, much knowledge has been accumulated about chromosome architecture and DNA conformation inside the cell. The characterization of DNA topoisomerases has been pivotal to this type of studies. As for transcription, the majority of the accumulated evidences has pointed to their role in the elongation process (i.e., in the removal of the stress generated during the tracking of RNA polymerase and of associated proteins along the DNA) [8,22–25]. On the other hand, it is possible that these enzymes play a major role in the control of transcription initiation. The maintainance of the active or of the inactive state of expression of a defined set of genes still needs to be elucidated from a mechanical point of view; this problem represents a major challenge for the understanding of differentiative and developmental processes at the molecular level.

Role of DNA Topoisomerase I in Gene Expression

Whether the nature of the involvement of DNA topoisomerases in the regulation of gene expression is direct or indirect is still controver-

sial. In *S. cerevisiae,* it has been reported that DNA topoisomerase I mediates a general transcriptional repression when cells stop exponential growth and approach the stationary phase [26]. The process is rather specific because not every gene is repressed. Moreover, some of the genes which are specifically activated in the stationary phase are more efficiently transcribed in a DNA topoisomerase I deficient strain. The explanation provided is speculative and is based on the modulation of chromatin architecture [26].

In another study in yeast, it was shown that transcription of ribosomal minigenes on extrachromosomal plasmids is greatly stimulated in a top1-top2 strain (where the DNA topoisomerase I TOP1 gene is deleted and the TOP2 gene contains a temperature sensitive mutation) [27]. Transcription initiation by RNA polymerase I is stimulated by negative superhelicity, as demonstrated in reactions with extracts from the double mutant strain [27].

A report from our laboratory shows a correlation between DNA topology and RNA polymerase II–dependent gene expression in yeast in vivo. The alcohol dehydrogenase II (ADH2) gene, but not the ADH1, is more efficiently expressed in a DNA topoisomerase I mutant strain, as compared to its isogenic wild type [28]. At the same time, a substantial increase in linking number is observed after the addition of ethanol in the wild type strain but not in the top1 mutant [28]. These and similar observations have led to the conclusion that DNA topoisomerase I controls the kinetics of promoter activation in yeast in vivo. As previously shown, the accumulation of positive DNA supercoils in yeast chromatin results in a cessation of transcription [29].

As in yeast [28], also RNA synthesis by human RNA polymerase II is sensitive to the conformation of the DNA template: in vitro transcription from the immunoglobulin heavy chain (IgH) promoter is enhanced by increasing negative superhelicity in reactions containing only TATA-binding protein (TBP), TFIIB, and RNA polymerase II [30]. By contrast, RNA synthesis from the same promoter in the linear form is dependent also on the presence of TFIIF, TFIIE, TFIIH, and of a fraction containing TFIIA and TFIIJ. It is suggested that the free energy of supercoiling promotes the formation of an open complex for initiation of transcription, thus avoiding the requirement of additional factors [30].

GENE-SPECIFIC ACTION FOR DNA TOPOISOMERASE I

A common feature of these and other [31] reports is that the transcription of a defined set of genes is sensitive to variations of DNA structure or of the DNA topoisomerases function. The same occurs in prokaryotes: only defined promoters are influenced by mutations in the genes coding for DNA topoisomerases or by environmental changes affecting the degree of DNA supercoiling inside the cell.

This specificity is not surprising if one considers that each promoter has its own specific DNA sequence and therefore its own DNA conformational information. Its information must satisfy the requirements for binding and for activation of the defined set of proteins (both the ubiquitous and the specific transcription factors) acting at that particular promoter. In other words, the structure of each promoter has to be finely modulated in order to ensure controlled gene expression. This concept is inherent to the architecture of the eukaryotic chromosomes that, like the prokaryotic ones, are organized in circular domains (loops) that obey the rules of topology, independent from each other because of the attachment to the nuclear matrix. Interestingly, the sites of DNA anchorage contain recognition sequences for DNA topoisomerase II, one of the major components of the chromosomal scaffold, and the matrix associated regions (MAR) are rich in enhancers.

A direct involvement of DNA topoisomerase I in the control of gene expression was demonstrated recently: the *h*uman enzyme is required for maximal activity of specific promoters and mediates the repression of basal transcription by interacting with the TATA-binding protein (TBP) subunit of TFIID [33]. According to the proposed model, *h*DNA topoisomerase I is loaded onto the transcription complex via the TFIID complex; in the absence of the activator, this interaction results in repression of transcription, whereas in the presence of the activator DNA topoisomerase I is translocated to the elongating complex and removes the superhelical tension induced by the elongation process [33]. Strikingly, the relaxing activity of *h*DNA topoisomerase I is dispensable for transcriptional repression, as demonstrated by using an enzyme with a mutation in the catalytic site. This means that a completely new role can be attributed to

this enzyme, previously known only for its nicking-closing activity.

Although its involvement in the control of gene expression seems to be conserved in evolution, the gene for DNA topoisomerase I is not essential for cell viability in yeast, whereas the homologous gene in Drosophila is essential for development [34]. Different roles for DNA topoisomerase I in gene regulation might have developed during evolution.

Most interesting is the consideration that DNA topoisomerases, enzymes known to have a general role in the control of DNA conformation and of chromosome architecture, are indeed involved in the regulation of specific promoters.

In a very similar manner histone proteins, known to have a general role in chromatin organization, act specifically at defined promoters [35]. Histone and DNA topoisomerase I have similar topological requirements (they appreciate curves and stress), they both influence DNA conformation and topology, and both regulate transcription in a general and in a gene-specific manner. The whole matter is still quite controversial; nevertheless, it can be safely stated that the involvement of DNA structure and topology in the control of gene expression is not an exotic topic anymore.

ACKNOWLEDGMENTS

This work was supported by Piani Finalizzati Ingegneria Genetica e Biotecnologie (CNR, Italy).

REFERENCES

1. Almer A, Hörz W (1986): EMBO J 5:2681–2687.
2. Almer RH, Hinnen A, Hörz W (1986): EMBO J 5:2689–2696.
3. Piña B, Barettino D, Truss M, Beato M (1990): J Mol Biol 216:975–990.
4. Piña B, Brüggemeier V, Beato M (1990): Cell 60:719–731.
5. Richard-Foy H, Hager GL (1987): EMBO J 6:2321–2328.
6. Germond JE, Hirt B, Oudet P, Gross Bellard M, Chambon P (1975): Proc Natl Acad Sci USA 72:1843–1847.
7. Freeman LA, Garrard WT (1992): Crit Rev Eukaryotic Gene Expression 2:165–209.
8. Liu LF, Wang JC (1987): Proc Natl Acad Sci 84:7024–7027.
9. Travers AA, Klug A (1987): Philos Trans R Soc Lond [Biol] 317:537–561.
10. Turnell WG, Travers AA (1992): Methods Enzymol 212:378–400.
11. Negri R, Costanzo G, Venditti S, Di Mauro E (1989): J Mol Biol 207:615–619.
12. Costanzo G, Di Mauro E, Salina G, Negri R (1990): J Mol Biol 216:363–374.
13. Buttinelli M, Di Mauro E, Negri R (1993): Proc Natl Acad Sci USA 90:9315–9319.
14. Meersseman G, Pennings S, Bradbury EM (1992): EMBO J 11:2951–2959.
15. Camilloni G, Di Martino E, Caserta M, Di Mauro E (1988): Nucleic Acids Res 16:7071–7085.
16. Camilloni G, Di Martino E, Di Mauro E, Caserta M (1989): Proc Natl Acad Sci USA 86:3080–3084.
17. Caserta M, Amadei A, Di Mauro E, Camilloni G (1989): Nucleic Acids Res 17:8521–8532.
18. Camilloni G, Caserta M, Amadei A, Di Mauro E (1991): Biochim Biophys Acta 1129:73–82.
19. Perini R, Caserta M, Di Mauro E (1993): J Mol Biol 231:634–645.
20. Camilloni G, Della Seta F, Negri R, Ficca AG, Di Mauro E (1986): EMBO J 5:763–771.
21. Della Seta F, Camilloni G, Venditti S, Di Mauro E (1988): J Biol Chem 263:15888–15896.
22. Giaever GN, Wang JC (1988): Cell 55:849–856.
23. Brill SJ, Di Nardo S, Voelkel-Meiman K, Sternglanz R (1987): Nature 326:414–416.
24. Brill SJ, Sternglanz R (1988): Cell 54:403–411.
25. Stewart AF, Herrera RE, Nordheim A (1990): Cell 60: 141–149.
26. Choder M (1991): Genes Dev 5:2315–2326.
27. Shultz MC, Brill SJ, Ju Q, Sternglanz R, Reeder RH (1992): Genes Dev 6:1332–1341.
28. Di Mauro E, Camilloni G, Verdone L, Caserta M (1993): Mol Cell Biol 13:6702–6710.
29. Gartenberg MR, Wang JC (1992): Proc Natl Acad Sci 89:11461–11465.
30. Parvin JD, Sharp PA (1993): Cell 73:533–540.
31. Mizutani M, Ura K, Hirose S (1991): Nucleic Acids Res 19:2907–2911.
32. Jupe ER, Sinden RR, Cartwright IL (1993): EMBO J 12:1067–1075.
33. Merino A, Madden KR, Lane WS, Champoux JJ, Reinberg D (1993): Nature 365:227–232.
34. Lee MP, Brown SD, Chen A, Hsieh T (1993): Proc Natl Acad Sci 90:6656–6660.
35. Durrin LK, Mann RK, Kayne PS, Grunstein M (1990): Cell 65:1023–1031.