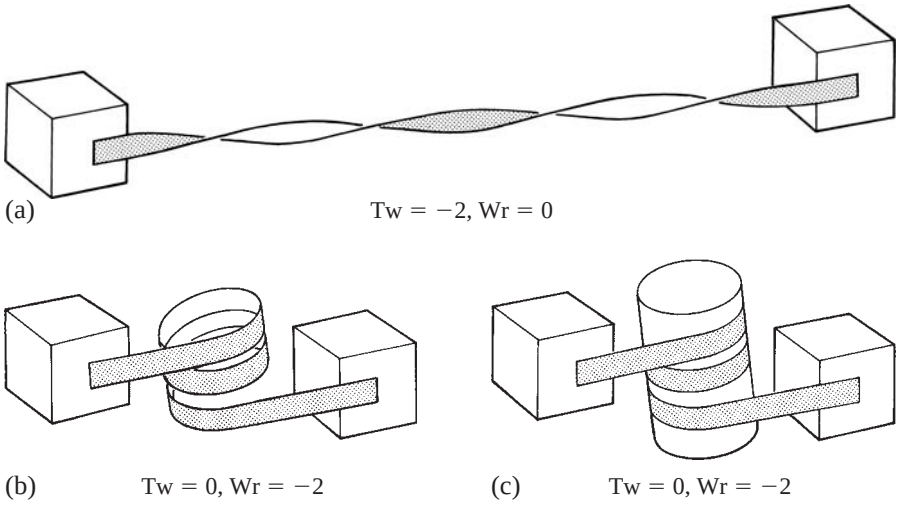


Figure 6.6 A highly twisted ribbon will collapse spontaneously into part of a toroidal supercoil. In (a), the two ends of the ribbon are held apart by their attachment to blocks, so that Tw = −2. In (b), the blocks move together so that the ribbon can collapse to Wr = −2. In (c), a cork or protein spool stabilizes the shape of the ribbon shown in (b).

coil in the interwound form of Fig. 6.2(b) also gives negative writhe. Is there some sort of mistake here? Surely the rule of crossovers should be consistent among different types of supercoil, such as interwound and toroidal? In fact, there is no mistake at all; the two sets of pictures have been derived unambiguously from physical experiments with underwound rubber rods or leather belts. The key point here is that we need a way of allocating a *sign* to any given crossover which we can see, in order to say unambiguously whether it contributes positively or negatively to writhe. This is not a trivial exercise, and so we have consigned it to Exercise 6.3.

We have now described two different kinds of supercoiling for DNA – toroidal and interwound. But what are the relative stabilities of these two forms? In other words, when will a DNA molecule be interwound, and when will it be toroidal? The interwound shape is usually very stable, and most underwound or overwound DNA molecules will naturally adopt an interwound shape, in the absence of other forces. But the proteins that associate with DNA in living cells can sometimes change the situation dramatically, and favor the toroidal over the interwound form by wrapping the DNA around themselves.

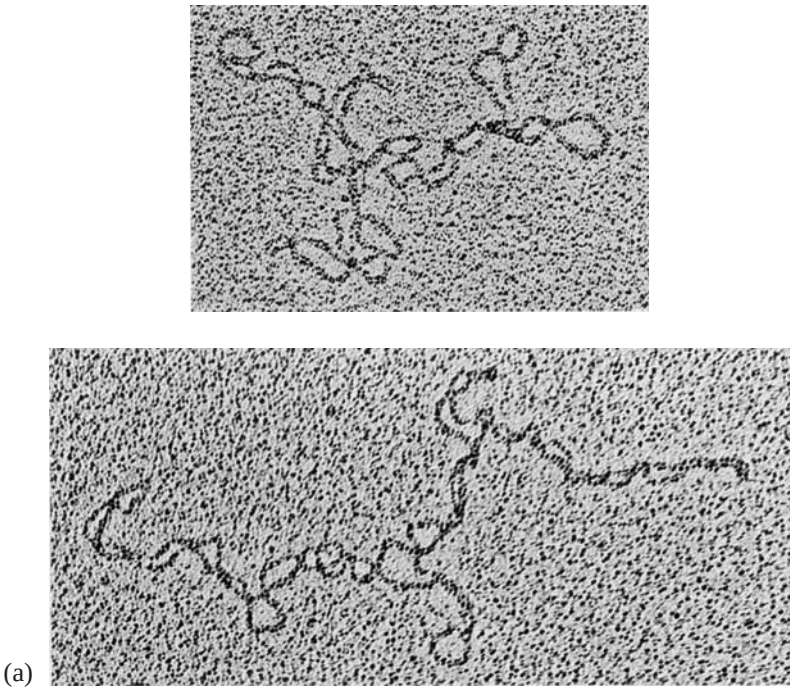
For example, consider the cork which has been inserted between the two turns of ribbon shown in Fig. 6.6(c). This cork represents a typical protein 'spool' around which the DNA can wrap, and around which it does wrap in a left-handed sense in the chromosomes of

most higher organisms on Earth. If the DNA or ribbon in Fig. 6.6(c) were to be cut free from the two blocks at either end, it would stay wrapped around the 'sticky' protein spool; whereas if it were cut free in the absence of a spool, as in Fig. 6.6(b), it would immediately spring back into a straight configuration. When we isolate DNA in the laboratory in pure form from any kind of cell or cells, at some point in the procedure we must strip off the proteins around which the DNA was originally wrapped, without breaking either of its two double-helical strands. In other words, we must remove the cork from the arrangement shown in Fig. 6.6(c), without cutting the DNA free from either of its two end-blocks. Naturally the 'naked' DNA will first spring out to the highly twisted form shown in Fig. 6.6(a), and then it can collapse into an interwound supercoil as shown in Fig. 6.5(e), because it has lost the curvature which stabilized the toroidal form. Therefore, we can expect to see highly interwound supercoils in the preparations of pure DNA which we make from living cells, after removal of various proteins. Incidentally, this is why DNA supercoils in Nature are usually underwound rather than overwound: the DNA always coils around proteins in the cell nucleus in the form of a left-handed toroidal spiral, giving negative Lk.

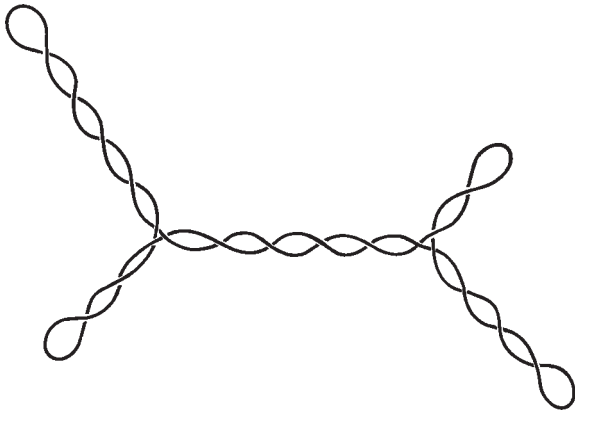
Some typical preparations of purified, protein-free DNA from bacteria are shown in Fig. 6.7(a), exactly as they appear in the electron microscope. These DNA molecules are circular forms of length 7000 base-pairs, and they are underwound on the average by 40 turns, i.e. $Lk = -40$. A linear, relaxed double helix of the same length would contain $7000/10.6 = 660$ turns of right-handed DNA, given a typical helical twist of 10.6 base-pairs per turn; so these DNA molecules contain just 620 turns and are underwound by $40/660 = 6\%$.

Note that the supercoils shown in Fig. 6.7(a) have a branched formation of the kind indicated schematically in Fig. 6.7(b). This does not in itself have much effect on either the topology or the energetics of the structure. Indeed, the branched structure could 'migrate' smoothly into an unbranched form that would be much longer overall, if conditions were right.

Assuming that these DNA molecules are all of the interwound form, can we determine their Lk, Wr, and Tw? A value for the linking number of $Lk = -40$ can be determined independently of the electron-microscope pictures, by studying the mobility of such DNA during gel electrophoresis: this method will be described in Chapter 9. An approximate value for the writhing number, $Wr = -36$, can be determined by counting the mean number of crossovers per DNA molecule, as seen in many pictures from electron microscopy such as those of Fig. 6.7(a). Finally, by subtraction, $Tw = Lk - Wr = -4$. Thus, the preferred interwound structure is



(a)



(b)

Figure 6.7 (a) Electron micrographs of negatively supercoiled, interwound DNA as prepared in pure form from *E. coli* bacteria. Each DNA plasmid or ring is 7000 base-pairs long, and has a mean Lk = −40. Courtesy of Christian Boles, Nick Cozzarelli, and James White; and from *Journal of Molecular Biology* (1990) **213**, 931–51. (b) Branched path of the interwound DNA shown in (a), in schematic form. Such branching has little effect on the parameters Lk, Tw, and Wr.

somewhat similar to the idealized shape shown in Fig. 6.5(e), since Wr = 0.9 Lk, and Tw = 0.1 Lk. In other words, the DNA which has been underwound finds it more favorable energetically to cross over itself repeatedly, than to alter its twist.

Some typical pictures of toroidal supercoils, where the coiling of the DNA has been stabilized by wrapping about proteins, will be shown in Chapter 7.

That concludes our survey of the physical properties of supercoiled DNA. We can now concern ourselves with its biological properties. Supercoiling is important in biology because it helps the DNA to unwind, so as to promote the synthesis of new RNA or DNA strands. As shown in Fig. 6.8, the various polymerase proteins that copy pre-existing DNA into new RNA or DNA must first unwind the DNA by one to two turns in the locations where they wish to act. This then enables them to 'read' the unpaired bases on one strand of the DNA, so that they can assemble a new strand according to the rules of Watson–Crick base-pairing. Sometimes it is not the polymerase that unwinds the DNA, but rather its 'helper' proteins. In any case, the DNA must be unwound or else accurate synthesis cannot proceed.

We said above that most of the missing turns in any negatively supercoiled DNA molecule are stored in the form of writhe Wr, whether by crossovers in an interwound supercoil, or by flat spirals in a toroidal supercoil. How, then, can supercoiling produce a reduction of twist Tw by one or two turns, as is needed for a polymerase protein to unwind DNA in various locations? Clearly, the DNA must be able to vibrate or fluctuate in solution, as a kind of Brownian movement, from shapes with high writhe to shapes with high twist. For example, an interwound supercoil might vibrate from the shape shown in Fig. 6.5(e) to any of the shapes shown in Fig. 6.5(d), (c), or (b), in order to generate twist. Similarly, a toroidal supercoil might vibrate from the shape shown in Fig. 6.6(b) to that shown in Fig. 6.6(a).

Unfortunately, we have few direct experimental data today, which might indicate how a DNA molecule fluctuates in solution over a large scale. We know, through probing for single-stranded regions using enzymes and chemicals, that negatively supercoiled DNA

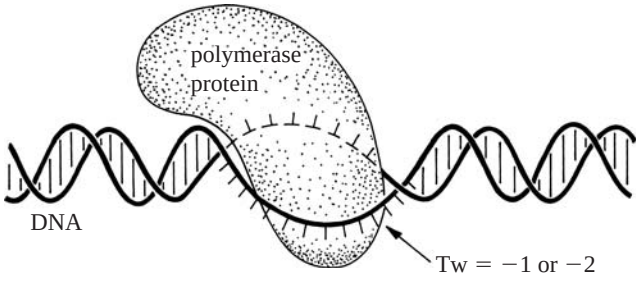


Figure 6.8 Untwisting of DNA by a typical polymerase protein, here shown schematically. Usually the twist of the DNA must be reduced by one or two turns in order for an RNA polymerase molecule to function.

vibrates much more efficiently than relaxed DNA to yield negative Tw; and we know also that many genes require negative supercoiling in order to be transcribed by RNA polymerase; but we do not know how DNA changes its shape over a large scale, to produce vibrations that lead to the generation of twist. Perhaps these involve changes in the local shape of the DNA from a right-handed supercoil to a plane curve, or from a plane curve to a left-handed supercoil, as described in Chapter 5. But all we have today are a great many lines of indirect evidence to suggest what might be going on. Furthermore, our indirect data are limited to observations about bacterial genes, because the genes in higher organisms are so poorly understood that one cannot draw any firm conclusions about how they work.

First, we have the observation that many different bacterial genes will synthesize more RNA, if the DNA in their 'promoter' region is appropriately curved due to its base sequence, than if this DNA is inappropriately curved or straight. Indeed, the most transcriptionally active genes in the bacterium *Escherichia coli* almost invariably have a region of curved DNA preceding the promoter. The structure of a typical bacterial gene is shown in Fig. 6.9(a). There we can see that an RNA polymerase protein starts to make messenger-RNA, just upstream of the long segment of DNA that codes for protein. The RNA copy of DNA then travels from the bacterial chromosome to

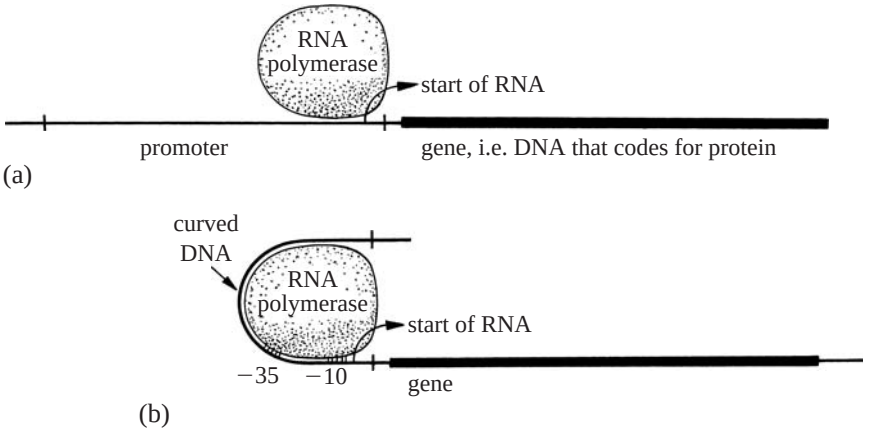


Figure 6.9 Highly schematic pictures of the structure of a typical bacterial gene, and its interaction with RNA polymerase. In (a), the polymerase protein binds just upstream of the DNA that codes for protein, and within the region of the 'promoter'. In (b), the presence of curved DNA within a promoter helps the polymerase to bind more tightly to the DNA, and hence to synthesize more RNA chains. Specific contacts between the protein and the DNA cluster in two regions that lie 35 and 10 base-pairs, respectively, upstream of the start-point for making RNA. The DNA unwinds at the −10 region (often of sequence TATA) to let the polymerase function as in Fig. 6.8.

the protein-making machinery, or ribosome, where the series of bases A, U, C, and G along its length specifies the synthesis of some protein with a certain number and ordering of amino acids. More protein is synthesized if there are more RNA molecules of a certain kind, and the number of RNA molecules depends in turn on how often RNA polymerase can initiate the synthesis of new RNA chains at any promoter.

Why should the curvature of DNA within a promoter affect how often RNA polymerase will start to make RNA? It makes no sense, if we consider the problem just in *one dimension* as shown in Fig. 6.9(a). It begins to make sense in *two dimensions*, when we consider that the curved DNA might wrap around the polymerase protein, as shown in Fig. 6.9(b), and so help it to bind at the promoter. In fact, there is considerable physical evidence that the *E. coli* RNA polymerase can bind at least up to 160 base-pairs, i.e. as much as a nucleosome, at a promoter. Most of this DNA is in front of the transcription start-site. When a polymerase approaches a promoter, it first probes for the identities of bases in two regions of the promoter called '−35' and '−10', as indicated in Fig. 6.9(b). Then it binds about 100 base-pairs of DNA upstream of the −35 region, and only then does it untwist the DNA in the promoter region to initiate transcription. Most importantly, binding of the DNA occurs best when it is negatively supercoiled; which suggests that it is wrapped onto the polymerase in a left-handed sense. Recent physical evidence for this wrapping of DNA around *E. coli* RNA polymerase seems compelling. For example, studies by Claudio Rivetti and colleagues using a combination of atomic force microscopy (see Chapter 9) and biochemical methods have shown that a typical promoter DNA wraps around the RNA polymerase protein by nearly 300° during the start of transcription, as shown in Fig. 6.10. Various protein-to-DNA contacts are necessary to stabilize that extensive curvature; but they represent only part of the full picture.

In this situation how could DNA supercoiling help the DNA in the −10 region to unwind? Now, when the DNA is free in solution the problem is relatively straightforward. Consider first the toroidal configuration of DNA in *three dimensions.* One turn of a left-handed, toroidal supercoil would yield $Wr \approx -1.0$ turn if it were straightened out as in Fig. 6.6(b) and (a). Thus, due to thermal fluctuations in solution, this DNA will vibrate fairly often into an extended form with $Tw \approx -1.0$ turn. In a free, highly supercoiled DNA this rapid interconversion of writhe and twist will result in transient strand separation at the most sensitive sequences, particular those containing TATA. However, when the DNA is wrapped around a protein such as RNA polymerase, it is constrained. Thus, if the

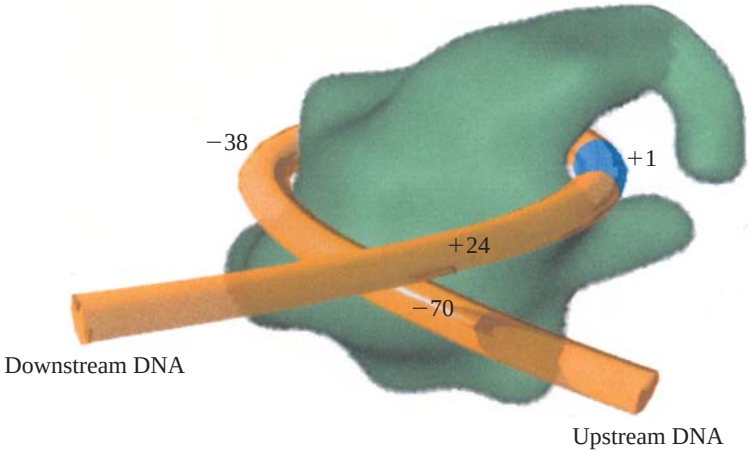


Figure 6.10 A model for the trajectory of promoter DNA around *E. coli* RNA polymerase, obtained by atomic force microscopy and biochemical methods. The DNA curves by about 300° in a negative (i.e. left-handed) supercoil; and the start-site for transcription is shown in blue. Courtesy of Claudio Rivetti.

wrapping of DNA about RNA polymerase could conceivably help the polymerase to unwind DNA near the start of the gene, i.e. in the −10 region, any change in shape of the bound supercoil comparable to the fluctuations of a free DNA molecule would be focussed. Alternatively, the energy of supercoiling could transmit a torque to the −10 region, by inducing a change in the polymerase protein itself.

Henri Buc and colleagues have shown that when RNA polymerase binds to a promoter without untwisting the DNA in the −10 region, one negative supercoil is constrained, presumably as writhe. However, the subsequent separation of the DNA strands around the transcription start-site increases the number of supercoils constrained to nearly 2: which shows that this step does not simply involve a direct conversion of writhe into twist, but that instead additional unwinding takes place. One possible solution to this paradox is to propose that the initial small untwisting in the −10 region could indeed be driven by the wrapped DNA, while the subsequent expansion of this untwisted region to encompass the transcription start-point could involve a concerted change in polymerase structure.

Furthermore, there is known to be a wide variety of proteins called 'activators' in bacteria, that seem to make genes work better in the following way: they bind to the DNA upstream of the polymerase, and curve it by up to 180°. It used to be thought that these activator proteins might simply stick to the polymerase, thereby helping it to bind the promoter as shown in Fig. 6.9(b). While this may still be true in

part, more recent data have shown three things. First, the activator proteins do not have to occupy any precise location along the DNA relative to the polymerase, so long as they curve the DNA in a correct direction. Second, some proteins that curve the DNA can act as 'repressors' of gene activity in one location, by binding competitively to the same piece of DNA as that preferred by RNA polymerase; then, later they can act as 'activators' of gene activity, when moved to a new location upstream, further from the gene. Third, protein-free DNA can furnish similar activation of genes, if this DNA is appropriately curved on account of its base sequence. In other words, although the activators help the polymerase to wrap the DNA, the polymerase can do this by itself if the DNA is curved, or negatively supercoiled.

Note

1. See Appendix 1.

Further Reading

Asayama, M., Kato, H., Shibato, J., Shirai, M., and Ohyama, T. (2002) A curved DNA structure in the upstream region of light-responsive genes: its universality, binding factor, and function of cyanobacterial *psbA* transcription. *Nucleic Acids Research* **30**, 4658–66. A biological role for DNA curvature in the promoters of certain genes in cyanobacteria (ancient photo-synthetic bacteria).

Benhoff, B., Yang, H., Lawson, C.L., Parkinson, G., Liu, J., Blatter, E., Ebright, Y.W., Berman, H.M., and Ebright, R.H. (2002) Structural basis of transcription activation: the CAP-α CTD-DNA complex. *Science* **297**, 1562–5. Promoter DNA curves sharply about CAP protein, and also adheres nearby to the α subunit of *E. coli* RNA polymerase.

Berger, J.M., Gamblin, S.J., Harrison, S.C., and Wang, J.C. (1996) Structure and mechanism of DNA topoisomerase II. *Nature* **379**, 225–32. The X-ray crystal structure of topoisomerase II from yeast, which can break and rejoin the DNA so as to change Lk.

Boles, T.C., White, J.H., and Cozzarelli, N.R. (1990) The structure of plectonemically supercoiled DNA. *Journal of Molecular Biology* **213**, 931–51. Electron microscope pictures of interwound supercoils as in Fig. 6.7.

Bramhill, D. and Kornberg, A. (1988) Duplex opening by *dnaA* protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. *Cell* **52**, 743–55. The transient unwinding of DNA as induced by its wrapping about proteins, near a bacterial origin of replication.

Cerf, C. and Stasiak, A. (2000) A topological invariant to predict the three-dimensional writhe of ideal configurations of knots and links. *Proceedings of the National Academy of Sciences, USA* **97**, 3795–8. A novel quantization of writhe Wr in certain knotted systems.

Coleman, B.C. and Swigon, D. (2000) Theory of supercoiled elastic rings with self-contact and its application to DNA plasmids. *Journal of Elasticity* **60**, 173–221. A detailed study of DNA supercoiling in terms of an elastic ring model of finite thickness.

Collis, C.M., Molloy, P.L., Both, G.W., and Drew, H.R. (1989) Influence of the sequence-dependent flexure of DNA on transcription in *E. coli*. *Nucleic Acids Research* **17**, 9447–68. The main part of the binding site for RNA polymerase can be curved DNA.

Crawford, L. and Waring, M. (1967) Supercoiling of polyoma virus DNA measured by its interaction with ethidium bromide. *Journal of Molecular Biology* **25**, 23–30. First demonstration that ethidium bromide can change the structure of supercoiled DNA, by altering the local twist of the double helix in many places.

Heggeler-Bordier, B., Wahli, W., Adrian, A., Stasiak, A., and Dubochet, J. (1992) The apical localization of transcribing RNA polymerases on supercoiled DNA prevents their rotation around the template. *EMBO Journal* **11**, 667–72. *E. coli* RNA polymerase induces a strong curve of 180° in the DNA, so strong that its binding site on DNA often becomes the curved end-loop of an interwound supercoil.

Kumar, A., Grimes, B., Fujita, N., Makino, K., Malloch, R., Hayward, R., and Ishihama, A. (1994) Role of the sigma-70 subunit of *E. coli* RNA polymerase in transcription activation. *Journal of Molecular Biology* **235**, 405–13. A careful study of the roles of −35 and −10 regions in bacterial promoters.

Lutter, L.C., Halvorsen, H.R., and Calladine, C.R. (1996) Toplogical measurement of protein-induced bend angles. *Journal of Molecular Biology* **261**, 620–33. A new method to determine with high accuracy, and in solution, the bending and twisting angles induced in DNA by a bound protein.

Ohyama, T. (2001) Intrinsic DNA bends: an organizer of local chromatin structure for transcription. *Bioessays* **23**, 708–15. A good review of DNA curvature, negative supercoiling, and their roles in RNA synthesis.

Schnos, M., Zahn, K., Inman, R.B., and Blattner, F.R. (1988) Initiation protein induced helix destabilisation at the origin: a prepriming step in DNA replication. *Cell* **52**, 385–95. The curvature of DNA around proteins at a viral origin of replication causes transient unwinding of the DNA, if it is negatively supercoiled in a test-tube.

Stump, D.M., Fraser, W.B., and Gates, K.E. (1998) The writhing of circular cross-sectional rods: undersea cables to DNA supercoils. *Proceedings of the Royal Society of London* **A 454**, 2123–56. A balanced-ply elastic model for DNA supercoiling.

Vinograd, J., Lebowitz, J., Radloff, R., Watson, R., and Laipis, P. (1965) The twisted circular form of polyoma viral DNA. *Proceedings of the National Academy of Sciences, USA* **53**, 1104–11. The earliest recorded work on DNA supercoiling.

Yan, H., Zhang, X., Shen, Z., and Seeman, N.C. (2002) A robust DNA mechanical device controlled by hybridization topology. *Nature* **415**, 62–5. Design of a nanoscale rotary device in which DNA topology can control structure.

Zaychikov, E., Denissova, L., Guckenberger, R., and Heumann, H. (1999) *E. coli* RNA polymerase translocation is accompanied by periodic bending

of the DNA. *Nucleic Acids Research* **27**, 3645–52. Curvature of DNA about RNA polymerase while it is engaged in making RNA.

Bibliography

Amouyal, M. and Buc, H. (1987) Topological unwinding of strong and weak promoters by RNA polymerase: a comparison between the *lac* wild-type and UV5 sites of *E. coli*. *Journal of Molecular Biology* **195**, 795–808. They find a loss of linking number but no loss of base-pairs on the initial binding of RNA polymerase to DNA; which implies that this protein induces negative writhe before it unwinds the double helix completely.

Bracco, L., Kothlarz, D., Kolb, A., Diekmann, S., and Buc, H. (1989) Synthetic curved DNA sequences can act as transcriptional activators. *EMBO Journal* **8**, 4289–96. Curved DNA can modulate gene activity in the cell.

Drew, H.R. and Travers, A.A. (1985) DNA bending and its relation to nucleosome positioning. *Journal of Molecular Biology* **186**, 773–90. DNA sequences from the upstream region of a bacterial promoter are shown to direct the same kind of curvature for DNA wrapped around RNA polymerase, or wrapped around the chromosomal histone proteins, or wrapped into a tight, protein-free circle.

Fuller, F.B. (1971) The writhing number of a space curve. *Proceedings of the National Academy of Sciences, USA* **68**, 815–19. Development of the concept of writhe for both interwound and toroidal supercoils.

Gartenberg, M.R. and Crothers, D.M. (1991) Synthetic DNA bending sequences increases the rate of *in vitro* transcription initiation at the *Escherichia coli* lac promoter. *Journal of Molecular Biology* **219**, 217–30. How the phasing of bent DNA can increase transcription of genes in the cell.

Pemberton, I.K., Muskhelishvili, G., Travers, A.A., and Buckle, M. (2002) FIS modulates the kinetics of successive interactions of RNA polymerase with the core and upstream regions of the tyrT promoter. *Journal of Molecular Biology* **318**, 651–63. How DNA near the promoter wraps around the bacterial polymerase, and how the −10 and −35 regions interact with it.

Rivetti, C., Guthold, M., and Bustamente, C. (1999) Wrapping of DNA around the *E. coli* RNA polymerase open-promoter complex. *EMBO Journal* **18**, 4464–75. Direct visualization of DNA curvature about RNA polymerase by means of atomic force microscopy.

Exercises

6.1 For this exercise you will need a substantial length (say about 1.5 m) of flexible rod or cord. An electric extension cord, with circular cross-section about 6 mm diameter, is ideal; or else you may use rubber tubing (provided it is not *curved* when relaxed) or even a plaited rope of the kind which is sold at boat shops. For the sake of convenience, we shall refer to all of these models as 'cords'.