# DNA TOPOLOGY

**Biochemistry 201**
**Advanced Molecular Biology**
**January 7, 2000**
**Doug Brutlag**

## Introduction

The double-stranded structure of DNA has many implications for biological function. Replication, at first appeared facilitated because genetic information is encoded twice in the DNA structure, once on each strand, however, the two parental strands must be separated and unwound to be copied.

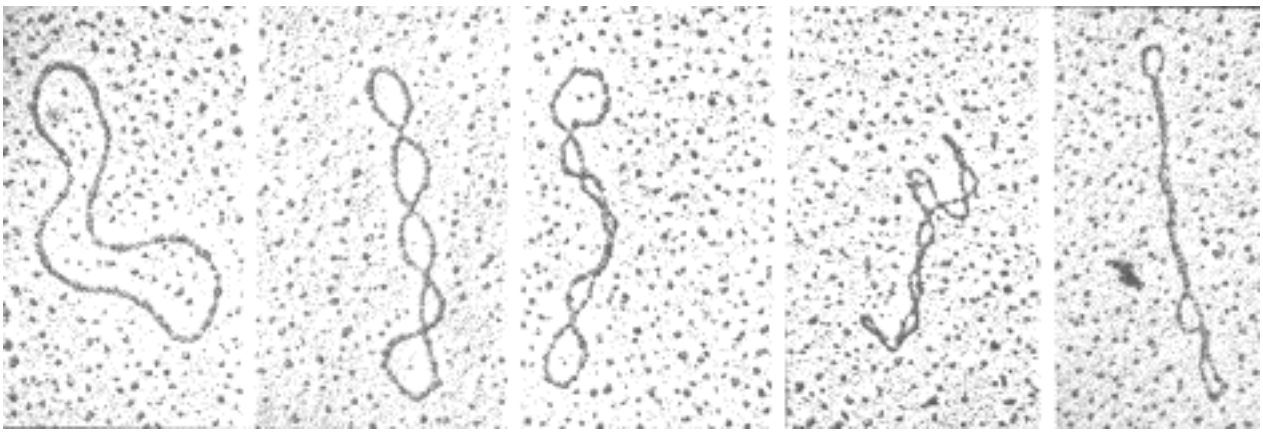
Transcriptional enzymes must decide which of two complementary strands contains the correct information to copy. Transcription also involves transient unwinding of the DNA helix in a local region in order to be able to copy one strand.

Metabolic events involving unwinding impose great stress on the DNA because of the constraints inherent in the double helix. Today we will discuss the topology of DNA and tomorrow we will cover enzymes that can alter the topological state of DNA without changing its primary structure. These enzymes are called DNA topoisomerases.

In addition to the requirement to unwind DNA for replication and for transcription, there is an absolute requirement for the correct topological tension in the DNA (super-helical density) in order for genes to be regulated and expressed normally.

## Supercoiled DNA

In 1965 Vinograd's group discovered that the circular DNA chromosomes isolated from small viruses like SV40 or polyoma were in a highly compact or folded conformation.



This supercoiling or writhing of circular DNAs was a result of the DNAs being underwound with respect to the relaxed form of DNA. There are actually fewer turns

in the DNA helix than one would expect given the natural pitch of DNA in solution (10.4 base pairs per turn).

When a linear DNA is free in solution it assumes a pitch which contains 10.4 base pairs per turn. This is less tightly wound than the 10.0 base pairs per turn in the Watson and Crick B-form DNA.

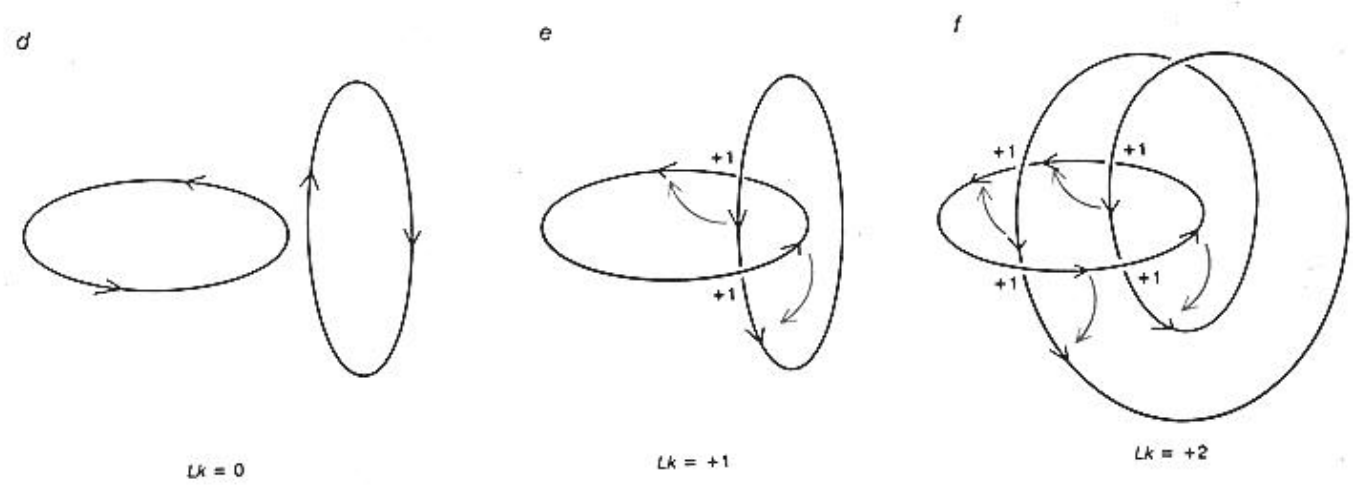
In order to understand the origin of supercoiling; imagine a linear DNA 5200 base pairs in length. If the DNA were in the B-form one would expect the two strands of the helix to be wrapped around each other 500 times (5200 bp / 10.4 bp / turn). Imagine a linear DNA in which the two ends become connected to form an open circle. This is referred to as a relaxed circular DNA. On the other hand, if the linear DNA were unwound 10%, say 50 turns, before its ends were joined, then the DNA molecule would be under stress. When the molecule is free in solution it will coil about itself in space as the two strands simultaneously twist about each other in order to return to equilibrium value of 10.4 base pairs per turn.

DNA that is underwound is referred to as negatively supercoiled. The helices wind about each other in a right handed path in space.

DNA that is overwound also will relax and assume a supercoiled conformation but this is referred to as a positively supercoiled DNA helix. Positively coiled DNA has its DNA helices wound around each other in a left-handed path in space.

## Linking, Twisting and Writhing

The total number of times one strand of the DNA helix is linked with the other in a covalently closed circular molecule is known as the linking number $L_k$.




1. The linking number is only defined for covalently closed DNA and its value is fixed as long as the molecule remains covalently closed.

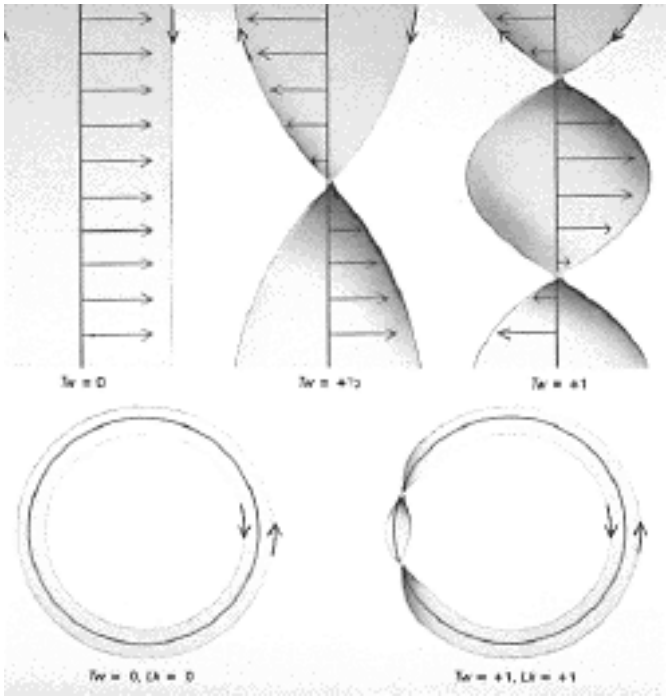
2. The linking number does not change whether the covalently closed circle is forced to lie in a plane in a stressed conformation or whether it is allowed to supercoil about itself freely in space.

3. The linking number $L_k$ of a circular DNA can only be changed by breaking a phosphodiester bond in one of the two strands, allowing the intact strand to pass through the broken strand and then rejoining the broken strand.

4. $L_k$ is always an integer since two strands must always be wound about each other an integral number of times upon closure.

The linking number of a covalently closed circular DNA can be resolved into two components called the twists $T_w$ and the writhes $W_r$.

$$L_k = T_w + W_r$$



The twists Tw are the number of times that the two strands are twisted about each other while the writhes Wr is the number of times that the DNA helix is coiled about itself in three-dimensional space.

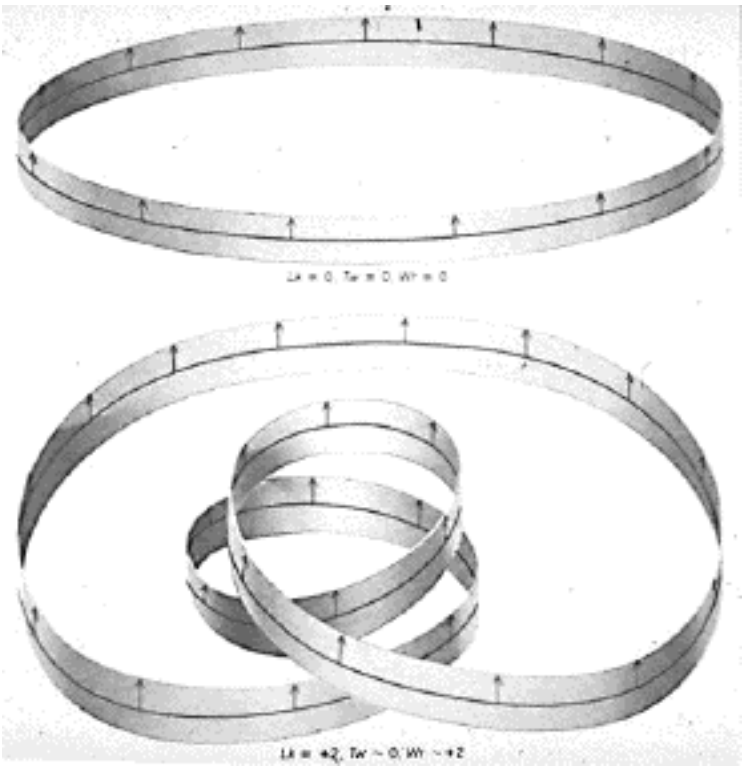
The twist and the writhe are not necessarily integers; indeed most often they are not. It is just their sum, the linking number, that is an integer. If we use an SV40 DNA molecule for example, which is precisely 5243 base pairs in length, we would find that:

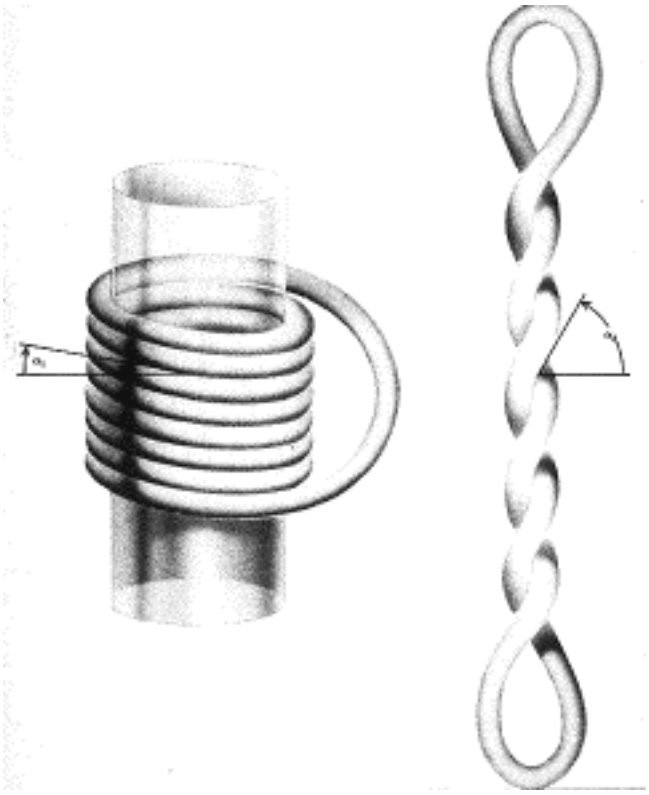
$$L_k = T_w + W_r$$
$$480 = 5243/10.4 - 24.13$$
$$480 = 504.13 - 24.13$$

Its length and its pitch in solution determine the twist of DNA. [$T_w$ = Length (bp) / Pitch (bp / turn)]

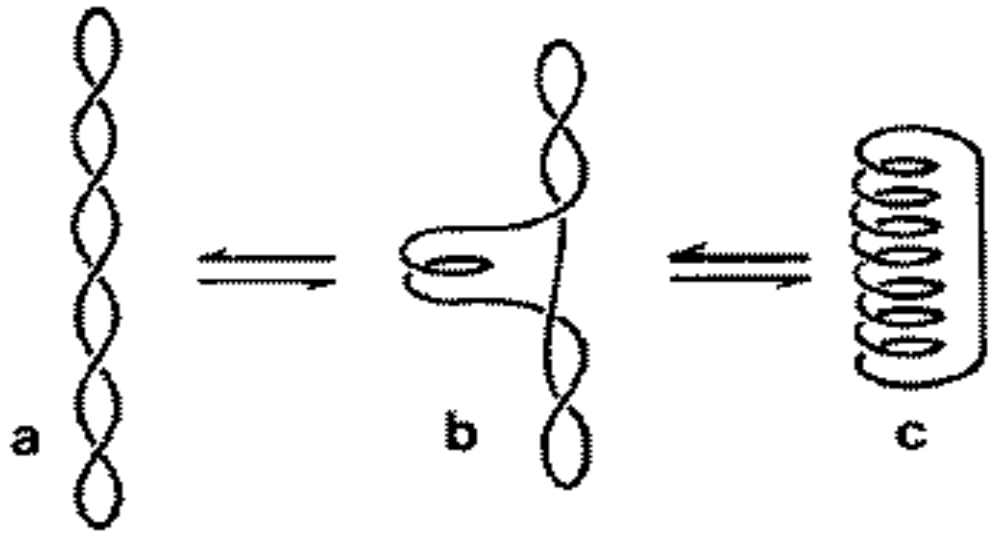
## DNA Topology

The twist and the linking number, determine the value of the writhe that forces the DNA to assume a contorted path is space. [$Wr = L_k - T_w$ ]





Unlike the Twist and the Linking number, the writhe of DNA only depends on the path the helix axis takes in space, not on the fact that the DNA his two strands. If the path of the DNA is in a plane, the Wr is always zero. Also if the path of the DNA helix were on the surface of a sphere (like the seams of a tennis ball or base ball) then the total Writhe can also be shown to be zero.

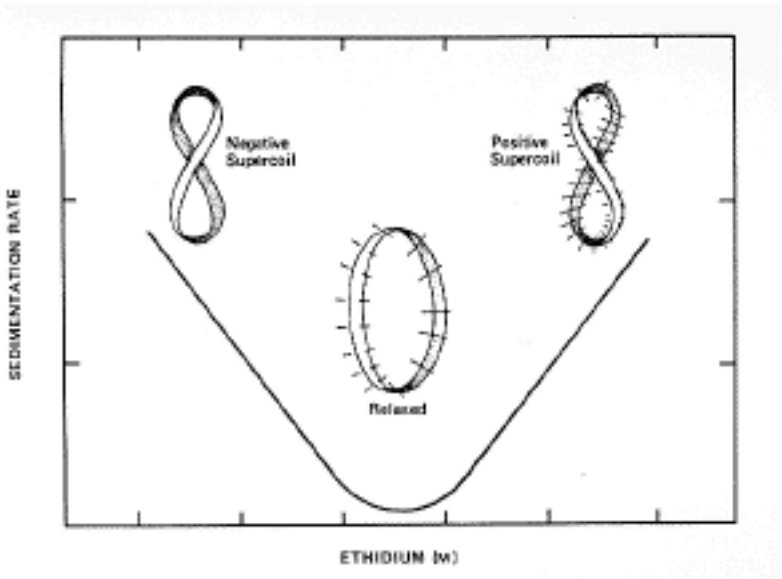



Writhes can also come in different forms. If a DNA molecule wraps around itself, then the writhes are known as supertwists. If a DNA molecule wraps around something else

(another molecule for instance) then the writhes are known as solenoidial writhe. In solution, the writhes can isomerize between the two types.

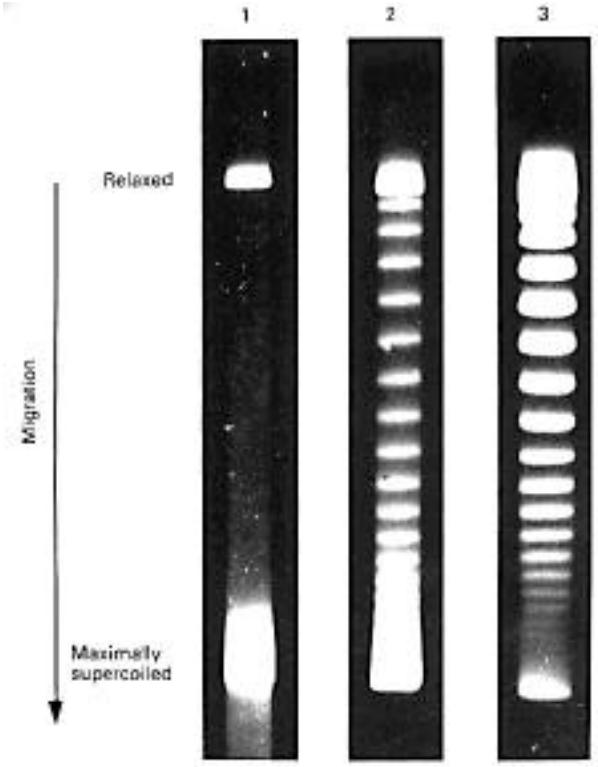
## Measuring Supercoils

The superhelical density of a circular DNA can be observed and measured in several ways, electron microscopy, sedimentation velocity, or by electrophoresis of DNA in a medium that provides frictional resistance.




The first quantitative method for measuring supercoiling was the sedimentation procedure. Since supercoiled molecules are more compact they sediment faster in a centrifuge than when they are relaxed.

A more elegant and simpler modern method for determining the number of superhelical turns in DNA is by electrophoresis in an agarose gel.

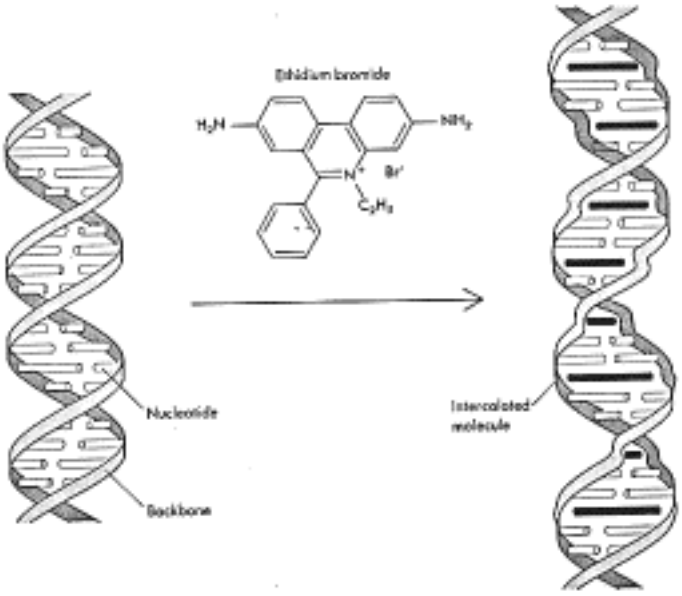


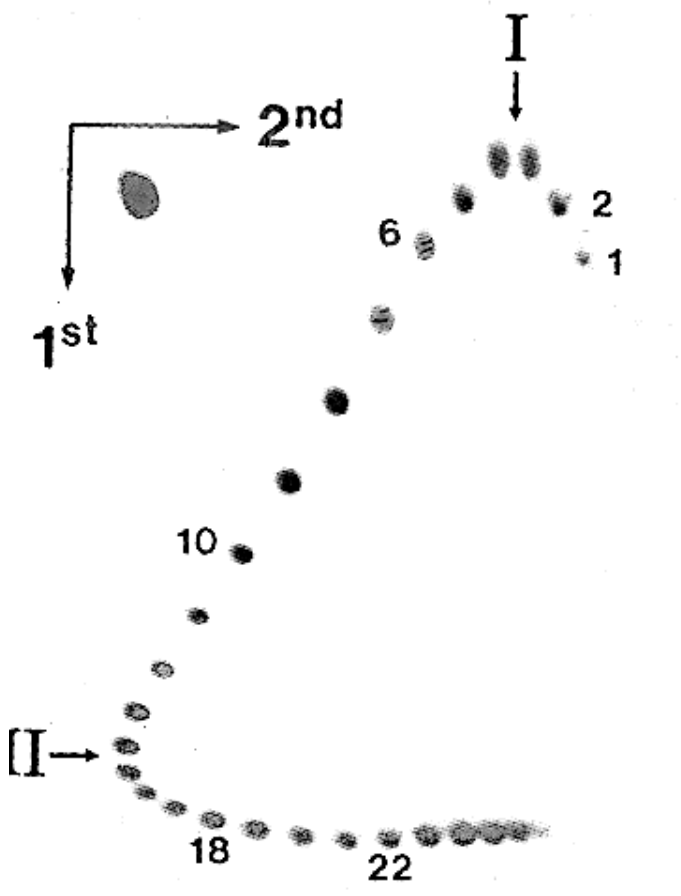

Supercoiled DNA migrates much more rapidly than does a relaxed molecule of the same length. DNA separates into discrete bands depending on the linking number.

Since DNAs resolved in this way differ from each other only in their topology, they are referred to as topological isomers, or topoisomers.

Molecules that differ by one unit in linking number can be separated by electrophoresis in agarose due to the difference in their writhe (that is due to difference in folding). The variation in linking number is reflected in a difference in the writhe. The variation in writhe is subsequently reflected in the state of compaction of the DNA molecule.

Neither of these methods by themselves can distinguish negatively and positively supercoiled DNA. Both forms sediment and electrophorese more rapidly than relaxed DNA. However by sedimenting or electrophoresing supercoiled DNA in the presence of an intercalating agent such as ethidium bromide or chloroquine, one can distinguish negatively supercoiled DNA from positively supercoiled DNA. When negatively supercoiled DNA binds an intercalating agent, the average pitch is reduced because the twist angle between adjacent base pairs on either side of the intercalating agent is reduced from 36° to as little as 10° of twist. This reduction of twist causes a compensatory increase in writhe in a covalently closed molecule. Hence a molecule that is initially negatively supercoiled will become more relaxed and a positively supercoiled molecule will become more twisted. By electrophoresing or sedimenting supercoiled DNA in increasing concentrations of an intercalating agent and knowing the affinity of the agent for DNA it is possible to calculate the writhe in the DNA.




It is also possible to measure the writhe and the sense of the write using intercalating agents in 2 dimensional gel electrophoresis experiments. By first electrophoresing DNA in one dimension in the absence of intercalating agents as above, and then adding an intercalating agent to partially increase the writhe in a positive sense and electrophoresing the DNA in a second dimension, it is possible to separate positive and negatively writhed DNA in the dimensions. It is also possible to get much better resolution of the highly twisted forms of DNA found in natural supercoiled DNA samples.

## Measuring the Pitch of DNA

Since the superhelical state of DNA is dependent on the pitch of DNA it became critical to have an accurate method to determine a DNAs pitch under different

experimental conditions. Since agarose gels can separate molecule differing by a single twist or writhe these were used to measure the change in pitch of DNA as a function of both temperature and sequence.

By relaxing circular DNA molecules at various temperatures and then electrophoresing the DNAs all at a single temperature both Vinograd's group and Wang's group determined that DNA tends to unwind by 0.012° per base pair per ° centigrade as it is heated.

While this does not seem like much change compared to the 36° rotation per base pair in the DNA helix, in even a short DNA molecule 10,000 base pairs in length, this amounts to an unwinding of 120° (1/3 turn) per ° centigrade. Heating a 10-kb circular DNA from 20° C to 35° C can reduce the overall twist by 5 full turns.

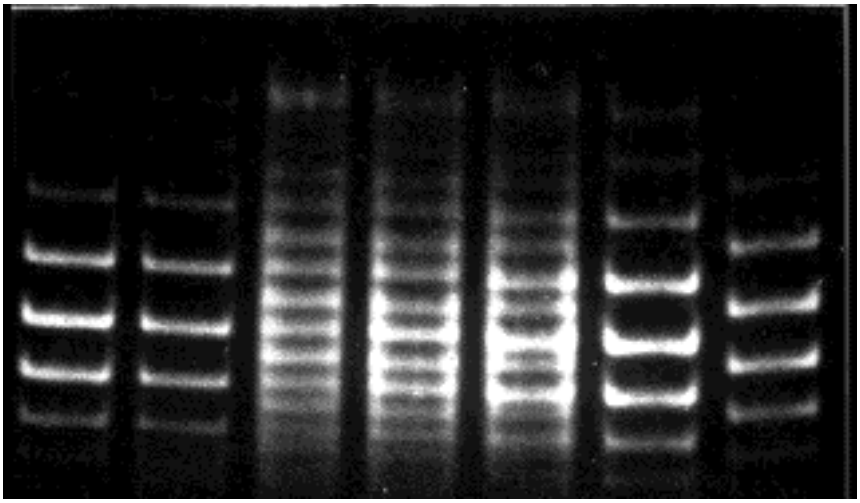


Table 2. Helical repeat $h$ of DNA calculated from measurements of DNA pairs with length differences >10 base pairs (bp)

| DNA pair | Δ length, bp | Shift | | Δ *Tw* deduced | *h* calc. |
|---|---|---|---|---|---|
| | | Observed | Corrected* | | |
| pTR193/pTR190 | 11 | 0.10 | 0.06 | 1.06 | 10.4 |
| pTR161/pTR199 | 25 | 0.49 | 0.40 | 2.40 | 10.4 |
| pTR161/pTR183 | 26 | 0.58 | 0.48 | 2.48 | 10.5 |
| pTR183/pTR182 | 27 | 0.69 | 0.59 | 2.59 | 10.4 |
| pTR161/pTR188 | 35 | 0.47 | 0.34 | 3.34 | 10.5 |
| pTR161/pTR193 | 47 | 0.65 | 0.48 | 4.48 | 10.5 |
| pTR161/pTR182 | 53 | 0.24 | 0.05 | 5.05 | 10.5 |
| pTR161/pTR190 | 58 | 0.78 | 0.57 | 5.57 | 10.4 |

* Corrected for length effect.

Wang also used gel electrophoresis to measure the absolute pitch of DNA in solution. By using a series of DNA molecules that differed from each other by a few base pairs (11, 25, 36 etc.) he was able to measure the amount of twist contributed by the extra base pairs. These measurements confirmed the X-ray crystal structure measurements of a pitch of DNA of 10.4 base pairs per turn of the helix (or 34.62° of twist per base pair). He also confirmed the dependence of the pitch on the sequence of DNA.

Klug further confirmed these measurements independently using a completely different method. By adsorbing small fragments of DNA to a mica surface and then treating the adsorbed DNA with DNAse I to nick the DNA, Klug showed that the DNA was accessible to nicking only on its side away from the mica surface and that this accessible region repeated every 10.6 base pairs, suggesting a pitch of that magnitude.

## References

**Bauer, W. R., Crick, F. H. C. and White, J. H. (1980). Supercoiled DNA. *Scientific American 243*, 118-133.**

Benham, C. J. (1985). Theoretical analysis of conformational equilibria in superhelical DNA. *Annu. Rev. Biophys. Biophys. Chem. 14*, 23-45.

Benham, C. J. (1987). The influence of tertiary structural restraints on conformational transitions in superhelical DNA. *Nucleic. Acids. Res. 15*, 9985-9995.

Crothers, D. M. (1998). DNA curvature and deformation in protein-DNA complexes: a step in the right direction [comment]. *Proc Natl Acad Sci U S A*, **95**(26), 15163-15165.

Cozzarelli, N. R. (1991). The structure and function of DNA supercoiling and catenanes. *Harvey Lect*, **87**, 35-55.

Crick, F. H. C., Wang, J. C. and Bauer, W. R. (1979). Is DNA really a double helix? *J. Mol. Biol. 129*, 449-457.

El Hassan, M. A. and Calladine, C. R. (1998). Two distinct modes of protein-induced bending in DNA. *J Mol Biol*, **282**(2), 331-343.

Giaever, G. N., Snyder, L., and Wang, J. C. (1988). DNA supercoiling *in vivo*. *Biophys. Chem. 29*, 7-15.

Smith, G,R, (1981). DNA supercoiling: Another level for regulating gene expression. *Cell 24*, 599-600.

Vologodskii, A. V. and Frank-Kamenetskii, M. D. (1992). Modeling supercoiled DNA. *Methods Enzymol*, **211**, 467-80.

Wang, J. C. (1974). Interactions between twisted DNAs and enzymes: The effects of superhelical turns. *J. Mol. Biol. 87*, 797-816.

Wang, J. C., and Giaever, G. N. (1988). Action at a distance along a DNA. *Science 240*, 300-304.

**Wang, J. C. (1979). Helical repeat of DNA in solution. Proc. Natl. Acad. Sci.. USA 76, 200-203.**

Wasserman, S. A., and Cozzarelli, N. R. (1986). Biochemical topology: applications to DNA recombination and replication. *Science 232*, 951-960.