Lecture 4

The linear double stranded DNAs that we have discussed so far in this class exists in a topologically relaxed state. The active DNA inside a cell is not relaxed DNA, but instead is supercoiled. This topological state of supercoiling is the next highest order level of DNA organization after the linear relaxed state.

In order for a DNA molecule to be supercoiled, its must be in a closed loop, with the ends fixed. Closure is achieved most often by joining the ends of an each DNA strand to form a circle. This, however is not the only way to close a loop; in a eukaryotic chromosome, for example loops are formed by holding the ends on a protein scaffold or by simply restricting the freedom of rotation of the ends of a long DNA molecule.

What is super coiling.

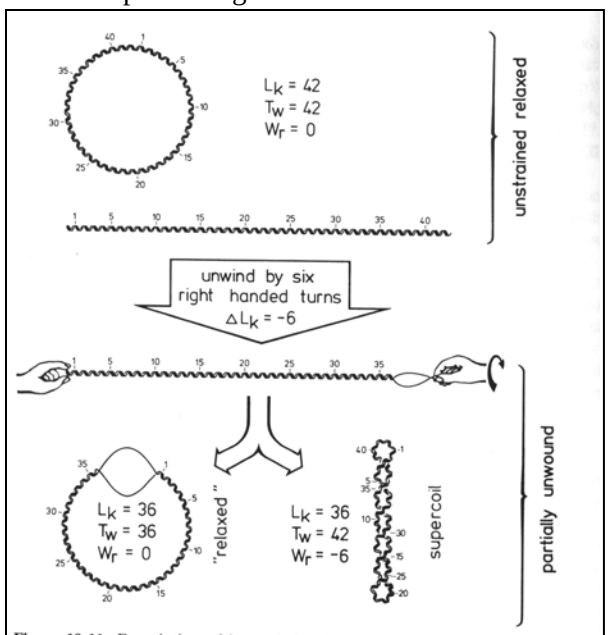

Figure 19-11. Description of interrelation between linking L_k, twisting T_w, and writhing W_r. Unstrained, relaxed B-DNA with 420 base-pairs has 42 turns of one strand wound about the other, $L_k = T_w = 42$ and $W_r = 0$ (top). If, now, the DNA is held tight at the left end, and the right end is rotated clockwise by six turns so that the double helix is partially *un*wound, L_k and consequently T_w reduce to 36. This DNA can again be circularly closed with $L_k = T_w = 36$ and $W_r = 0$; i.e., compared with the above picture, base-pairs of six turns are disrupted. Due to the tendency of DNA to retain the B-DNA form, however, T_w increases to the original $T_w = 42$ with 420 base-pairs. However, since L_k is a topological number, it is constant, $L_k = 36$ and in order to satisfy Eq. (19-1), the writhing must become $W_r = -6$, corresponding to a right-handed supercoil (superhelix) with six crossovers.

Figure 20

Picture a 420 bp piece of DNA duplex in the B-form. The number of helical turns in this linear DNA is 42 (42/10). Now we shall join the ends of this helix. This circular DNA is said to be relaxed **(FIGURE 20)**. Suppose now instead, we unwind the duplex by six turns before we join the ends. The resulting DNA can now fold into one of two different structures; one that contains 36 turns of B-helix and an unwound loop or alternatively, it can adopt a structure with 36 turns of B-helix and six turns of superhelix. The unwinding is taken up by allowing the previously unwound region to adopt the B-form and twisting the circle into a superhelical form. This is the supercoiled form. This structure is energetically favored over the one containing the unwound loop (WHY??). Notice that the superhelical form produced from unwinding the DNA is right-handed. This unwind produced, right-handed superhelix is called negatively supercoiled. On the other hand, superhelices formed by overwinding are left-handed and are said to be positively supercoiled. MOST DNA IN ORGANISMS IS FOUND TO BE NEGATIVELY SUPERCOILED.

Supercoiling play an important role on many life processes. For example, both the transcription of genes and replication of DNA require it to be unwound. Thus, negatively supercoiled molecules (right handed superhelices that result from joining underwound strands) are poised for these processes. In fact several antibiotics have been developed that kill by inhibiting the enzymes that modulate the degree of supercoiling in infectious organisms. We will briefly discuss these enzymes later.

DNA Topology

In order to better understand the relationship between the degree of supercoiling, and twist, we turn again to the mathematicians. An equation has been worked out to describe supercoiled DNA ribbons. The key

topolgical property of a circular DNA is its LINKING NUMBER $\mathbf{L_k}$. This quantity is defined as the number of times one strand of DNA winds around the other in the right-handed direction (since we are taking as our reference B-DNA). For the relaxed DNA shown in **FIGURE 20)**, the linking number is 42, for the one in C, the linking number is 36. The circles corresponding to the joining the ends of these two molecules is 42 and 36 respectively. NOTICE, THE SIZE OF THE DNA DID NOT CHANGE, HOWEVER THE LINKING NUMBER DID-CIRCLES OF THE SAME SIZE CAN HAVE DIFFERENT L_k. Molecules which are identical in length, and differ only in linking number are topological isomers or topoisomers. Moreover, since the end of a circle are fixed-THE LINKING NUMBER OF A CIRCULAR DNA CANNOT BE CHANGED-IT IS A TOPOLOGICAL QUANTITY-A PROPERTY OF THE CIRCULAR STATE. Since we are dealing with closed circles, the only way to form a circle is to match up the ends. THEREFORE, THE LINKING NUMBER OF A CIRCLE MUST BE AN INTEGER. Moreover, the only way to change the linking number of a circle (convert one topoisomer into another) is to CUT the strands. The linking number of a topoisomer is constant.

In a relaxed unstrained circle shown in **FIGURE 20b**, the linking number equals the twist (T_w). The twist is defined as the number of 360 degree turns the ribbon makes as it goes around the circle. The twist of a given topoisomer can differ, and can be non-integral, however, the linking number as we have said is constant for a particular topoisomer and, moreover IT MUST BE AN INTERGER. Twist is a metrical property and for DNA is limited within narrow bounds because B-form DNA does not stray very far from having 10 bp/turn. Thus for DNA, Tw is usually very close the the # of bp/10. Since topoisomers can have linking #'s that vary at least by two integers, as we have seen, these linking differences can be taken up by the unwinding DNA or, superhelix formation. Because of the structural demands of DNA, the various values of Lk a circle can have results not in changes in twist, but formation of superhelix. This super helix formation is decribed by the term writhe ($\mathbf{W_r}$). These three terms are obviously physically related as we have described, and are related mathematically by the equation:

$$L_k = T_w + W_r$$

As we mentioned before, L must be and integer once the DNA is closed and thus Tw and Wr can adopt any value, dependent upon the three dimensional shape of the DNA. For DNA, right handed twisting is positive as is righthanded rotations for Lk, but for right handed superhelices, the sign of Wr is negative.

Let's demonstrate how the concept of writhing (or superhelix formation) prevents the changes in DNA twist from causing alterations in the structure of the double helix. If circular DNA is confined to a planar arrangement, the changes in linking #

$$\Delta L_k = \Delta T_w + \Delta W_r$$

can only be compensated for by equivalent changes in twist, because W_r is held at zero. In this case, ΔT_w can be compensated for by smoothly modifying all helical turns, or since B-DNA likes to remain that way, by disrupting a few base pairs. If, on the other hand T_w is unchanged, then ΔT_w is zero and thus

$$\Delta L_k = \Delta W_r$$

that is the change in linking number is transformed almost entirely into writhe. Thus in the example given in **FIGURE 20c & d**, the DNA is twisted in a right handed sense (UNWINDING), i.e. REDUCTION of L_k, and therefore the change in ΔL_k is compensated by righthanded motion superhelix formation, or negative writhe.

As we said before, most DNA *in vivo* is negatively supercoiled. The degree to which a molecule is supercoiled can be expressed as the **specific linking difference** or **superhelical density**. This quantity gives a length independent measure of the number of supercoils per 10 base pairs. The formula:

$$\sigma = (L - L_o)/L_o$$

L_o is the linking number of the relaxed circular molecule and L linking number of the supercoiled molecule. For the DNA that is in the Figure, L_o is 25, L=23, thus σ= -.08. The superhelix density of DNA in cells is between -.05 to -.09. That means, the DNA is negatively supercoiled (i.e. right handed superhelices resulting from Unwinding).

Supercoiling and Hydrodynamics

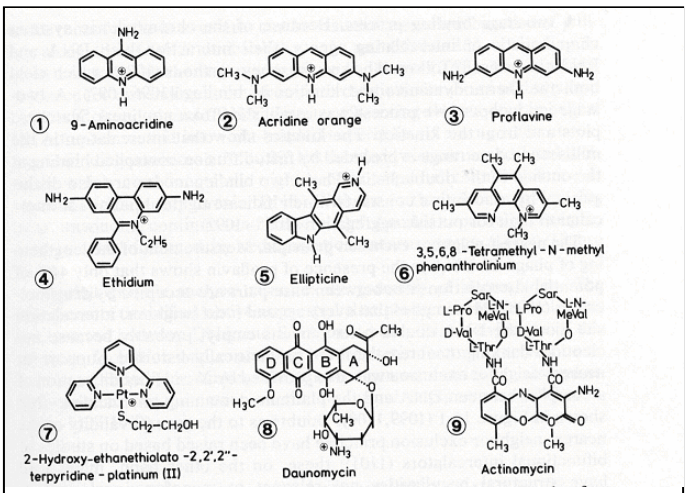

Figure 21

The effect of circularizing a molecule effectively halves the effective mean square radius. Thus, the molecule appears smaller by a number of experimental techniques, among them gel electrophoresis and density gradient centrifugation. Supercoiling a molecule further compacts its shape. This compaction is another reason why the DNA is a cell is supercoiled-it compacts it.

Intercalation means the insertion of a planar molecule (e.g. other bases, drugs) base between two others. This requires the spacing between the two bases to increase. Several dye molecules and antibiotic drugs insert themselves between base pairs. These intercalators are planar, aromatic molecules which bind to the DNA by essentially becoming part of the base pair stack **(FIGURE 21)**. Intercalative binding is, therefore, characterized by high binding affinities.

Intercalation changes the physical properties of the double helix. As the intercalator slides between the bases, their normal stacking interactions are disrupted as they now stack with the drug. This separation of the base pairs forces a severe distortion of the regular helical structure of the sugar phosphate backbone. Since the intercalation behaves nearly as an extra base pair, as the more intercalator is added, the greater is the apparent length of the molecule. The separation of the base pairs causes them to UNWIND, in order to accommodate the insertion.

If we start out with negatively supercoiled DNA and add intercalator, we further unwind the helix. In the case of ethidium bromide, the degree of unwinding is -26°/molecule bound. Since we are not changing the linking number of the plasmid, the changes in T_w are solely compensated for by an increase in writhe. Recall that negatively s.c. DNA has negative writhe so that an increase in W_r indicates that W_r-->0 as more intercalator is added. As the W_r-->0, the DNA loses its compact shape and thus appears "bigger" in gel or centrifugation experiments **(FIGURE 22)**. At even higher concentrations of intercalator, the T_w<L_k and thus, the W_r becomes positive, with the opposite screw sense (Positive supercoils [+W_r], left

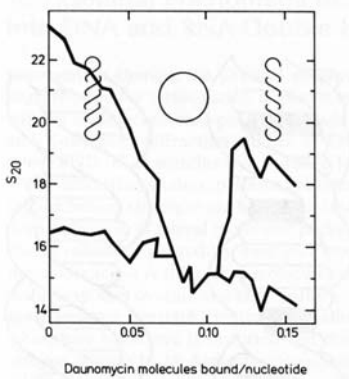

Figure 22

handed screw. This exercise illustrates the interrelation of these three quantities and the constraints imposed on circle-i.e., the L_k never changes.

For a change and much to your relief, the intercalation process does not cause a simple change in the back bone configuration. The sugars of B-DNA appear to remain in the C2'endo family. The major changes in the angles of the backbone occur at the phosphodiester linkages and at the χ-angle which now cause the bases to become strictly perpendicular to the sugar.

The enzymology of supercoiling

Inside of all cells there is a class of enzymes first discovered by Gellert and Wang in the early 1970's. These enzymes called topoisomerases regulate the supercoiling of DNA in a cell. They are called this because they can *isomerize*, one topoisomer (RECALL DEFINITION OF TOPOISOMER-DIFFERENT L_k) into another. There are at least two kinds of topoisomerases, Topo I and Topo II.

Topo I type topoisomerases relieve supercoiling, i.e., they relax supercoiled DNA. These enzymes do so without the input of energy. They act by cutting one strand of the supercoiled double helix, rotating one strand about the other and then reseal the strands. This reaction proceeds fueled only by the energy inherent in supercoiled DNA. More about the inherent energy later, but there is an interesting enzyme mechanisms question here, and that is how is the energy of the cleaved phosphodiester bond saved. Well it turns out that the cleaved strand of the DNA forms a covalent bond to a tyrosine-the same type of high energy linkage found in the phosphodiester backbone of DNA.

Topo II type topoisomerases function to put supercoils into DNA. The enzyme gyrase required for DNA replication is a type II topoisomerase. The introduction of supercoils into DNA requires the hydrolysis of ATP. Topo II enzymes function by making double stranded breaks in the DNA and rotating one strand past the other and rejoining them. SInce supercoiling is an active process, requiring energy in put, this indicates that supercoiled DNA is a high energy form of DNA. In fact the ---- increases with the square of the superhelix density.