DNA topology

1. Query: Why can supercoiled DNA move faster through an agarose gel during electrophoresis?- the answer that I have is that because it can constantly reorient itself, but I am not sure how it can reorient itself.

Supercoiling compacts DNA compared to the relaxed circle or linear DNA. Hence the delay for reorienting of molecules for passage through agarose gel is least for supercoiled molecules and highest for relaxed or nicked circular DNA. Hence the order of mobility is:

Supercoiled>>linear>nicked circular.

Depending on the length of the DNA molecule (supercoils will branch, as we discussed in class). The branching itself will slow down reorientation. However, branching also reduces the effective length, so overall it sort of equalizes things.

2. Query: The number of base pairs (bp) per turn changes when we compensate torsional strain with change in twist (Tw), so to avoid changing base pairs per turn, is the stress compensated by writhe (Wr) preferentially?

Yes, energetically, double stranded DNA prefers the B form structure with 10 base pairs per turn. So if the DNA is underwound (DNA turns removed from the relaxed state by cutting and joining strands), the torsional stress is accommodated primarily by writhe (crossing of the axis; or crossing double helical segments), so that the twist can be maintained as close to normal as possible (10 bp per turn).

[----unless you have some way of accommodating the reduced twist. This is what we saw in the case of ethidium bromide binding. Ethidium bromide binds to DNA (becomes part of the helix and unwinds the helix or reduces the twist between strands), as it does so. So the DNA molecule is negatively supercoiled (or unwound) in the context of the bound ethidium bromide. Since the change in the twist is stabilized by the ethidium bromide, the axis does not writhe. Here is a case of supercoiling being partitioned almost entirely into twist. This is the basis of why binding of ethidium bromide to negatively supercoiled DNA ‘apparently relaxes it (by changing writhe into twist). WHEN YOU REMOVE WRITHE (CROSSINGS OF THE AXIS), YOU ARE MAKING THE AXIS LESS AND LESS NON-PLANAR. YOU ARE APPROACHING THE APAPRENT RELAXED STATE].

In bacterial cells, you see DNA writhe in the form of plectonemic supercoils (right handed for negative supercoils; left handed for positive supercoils). In eukaryotic cells, you see it in the form of DNA wrapped around histones as solenoidal supercoils (left handed wrap for negative supercoils; right handed wrap for positive supercoils). By the thumb/index finger rule convention, the node sign is negative for both plectonemic and solenoidal negative supercoiling. The sign is positive for both types of positive supercoiling.

However, please remember that twist and writhe are inter-convertible. The average twist of DNA will change somewhat with buffer conditions, temperature etc. and accordingly writhe will also change (to maintain Tw plus Wr = Lk, which does not change unless you cut strands, add or remove DNA turns and then rejoin strands). As I said in class, change in twist alone or change in writhe alone are two extreme conformations with all sorts of intermediate conformations (with changes in twist as well as writhe) being possible. Many of these are highly transient, so by electrophoresis, you are looking at an averaged conformation, which is stable over a long period of time but highly dynamic over very short times.

If you remove 10 turns from a DNA, you could certainly imagine a conformation in which loss of 5 turns is converted to writhe (5 negative superhelical crossings of the axis), and the loss of the other five into twist. That is, the twist in the original DNA (total turns = n and total length = n x 10 bp in relaxed state) was 'n', and now the twist has changed to n-5. Or, the number of base pairs per turn are now greater than 10 = 10 x n divided by (n-5).

3. **Query**: When topoisomerases 'cut out' DNA, does this not affect the coding region? Is the answer 'no' because the topo is behind the fork?

Toposiomerase do not cut out (remove DNA). They act by transiently cutting DNA (single stranded cut for topo I and double stranded cut for topo II), passing DNA through the gate and covalently closing the cut strand. There is no change in the sequence of DNA (no change in the genetic information).

During replication, the topoisomerase acts ahead of the fork. The positive supercoils are built up in front of the fork to compensate for the underwinding of strands (negative supercoiling) behind it. The overwound DNA (positive supercoils) must be relaxed so that strands can be continuously unwound and the replication machine can move forward.

The first and second part of the question are not related. Both are conceptually incorrect. When a gene is going through replication or DNA is being relaxed by the action of topoisomerase within the gene, transiently the flow of information from it into RNA and protein will be interrupted. However, the time taken for the replication of a gene or the time taken for a round of relaxation by topoisomerase is quite small compared to the time required for a cell to replicate its entire genome, and divide into two cells (or become new cells). Thus the transient interruption has no effect on the overall cycle processes.

4. **Query**: You said that topo I had two ways to relieve strain? The first one is that it cuts one strand and then passes the strand through the gate and closes it, but what is the second one for topo I? (I have the one for topo II-it cuts two strands and brings the back one forward and changes the magnitude from positive to negative).

The mechanism I drew in class for topo I is by passing the uncut strand through the DNA gate and then closing the gate (strand sealing by a ligation type reaction). This puts back one turn of

Watson-Crick DNA into the negatively supercoiled substrate DNA. Two strand crossings, each corresponding to +1/2 linkage or a change in linking number of +1.

In the other mechanism, the cut strand rotates around the uncut strand and is then joined as in the previous mechanism. The end product is exactly the same, one new Watson-Crick DNA turn (delta Lk = +1). Since the negatively supercoiled DNA has a deficit of right handed turns (compared to the relaxed state), the cut strand rotates in the right handed sense to put back the missing turns in DNA.

5. Query: Superhelical Density- you were talking about E. coli, I believe it is -0.06, what is the purpose of calculating the superficial density- I remember you started off with the change of the Lk -Lk naught (Lk-Lk0) divided by Lk naught (Lk0) but I am not sure how this relates?

The super helical density is a convenient way of representing how underwound or overwound a DNA molecule is compared to its relaxed state. The linking number of the DNA under a given condition is denoted as Lk, the relaxed linking number of the molecule by Lk0. The difference in linking number is Lk - Lk0. This difference divided by Lk0 is the superhelical density. It is positive for overwound DNA. It is negative for underwound DNA. For perfectly relaxed DNA, it is zero (because Lk = Lk0; therefore, Lk - Lk0 = 0).

Normally the superhelical density of DNA inside the cell is approximately -0.06. That is for every 100 turns expected for the relaxed state, the actual number of turns in the DNA is only about 94 (Lk - Lk0 = -6).

6. **Query**: Could you explain how the binding of ethidium bromide changes the supercoiling of DNA?

EtBr unwinds DNA as it intercalates between bases. DNA unwinding is negative supercoiling. Since the unwinding angle is about 28-30 degrees per molecule of EtBr bound, about 12-14 molecules of EtBr will effectively unwind DNA by one turn (Change of Lk = -1). Hence, in a covalently closed circular DNA the binding of EtBr (or unwinding of DNA) must be compensated for by equivalent positive supercoiling. [Think of the replication fork movement. DNA is unwound behind the fork, but it is equally overwound in front of the fork.]

Imagine a supercoiled molecule with a deficit of n turns (delta Lk = -n). As we add EtBr, it introduces compensatory positive supercoils, which neutralizes the negative supercoils in the original molecule. When about 12 molecules of EtBr (or one negative turn worth of EtBr) is bound, one compensatory supercoil is introduced, or the apparent delta Lk of the DNA now is –n+1. Then as more EtBr binds, the apparent delta Lk becomes –n+2, -n + 3 etc., and at some point it will be –n + n = 0. Or the DNA will be relaxed, but only in the context of the bound EtBr. The true delta Lk is still –n, since we have not cut and joined strands. This can be shown by removing the bound EtBr by a process called dialysis (which allows small molecules to diffuse out through a semipermeable membrane). As soon as the EtBr is gone from DNA, it returns to its original delta Lk of –n.

Imagine a case where you continued to add EtBr even after the apparent delta Lk has become zero. Now the compensatory positive supercoils will accumulate and the DNA will have an apparent positive delta Lk. As you continue to add EtBr, the apparent delta Lk will go from 0 to +1, +2, +3 etc. imagine a state where you have added enough EtBr, so the DNA has an apparent delta Lk of +n.

If you again remove the EtBr by dialysis, the DNA will go back to its native state, delta Lk = -n.

Instead, let us now treat the DNA with topoisomerase. Topoisomerase can remove the n positive supercoils. But this apparently relaxed DNA has 2n negative turns worth of EtBr still bound to it (n to take the DNA from –n to 0, and another n to take it from 0 to +n). If you remove all the bound ethidium bromide by dialysis, the DNA will now have a delta Lk of -2n (-n plus –n). Or you have super-supercoiled DNA.

[Another way to think about EtBr binding is as follows. When EtBr binds to DNA, it unwinds strands, or introduces negative supercoiling. Recall that that this supercoiling can be partitioned into change in twist or change in writhe or both. EtBr changes twist (number of base pairs per turn of DNA), and does not change writhe. If you have a DNA circle (covalently closed) with one negative supercoil (Wr = -1) and it binds 12-14 molecules of EtBr, it will have acquired another negative supercoil. However, this one is due to the changed twist of DNA caused by the bound ethidium, and no writhe is added to the DNA. Yet, the newly added negative supercoil (due to the bound EtBr) must be compensated by a positive supercoil introduced in the DNA (Wr = +1). This positive super coli and the original negative supercoil will cancel each other (Wr = -1 + (+1) = 0). So the DNA has no net writhe, and therefore '***looks*** relaxed'. However, it is not '***really*** relaxed', because the bound EtBr has changed its twist. This will become evident if you remove the bound EtBr. The DNA now shows the negative supercoil by reverting to normal twist and changing its writhe to -1. Left to itself (that is, in the absence of intercalating agents such as EtBr), DNA adjusts to torsional strain in it by writhing (coiling of the axis) so that the normal twist of 10 bp per turn is maintained).