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Supplemental Information

Cellular Strategies for

Regulating DNA Supercoiling:

A Single-Molecule Perspective

Daniel A. Koster, Aurélien Crut, Stewart Shuman, Mary-Ann Bjornsti, and Nynke H. Dekker

Supplemental Information I. Gel electrophoresis

The charge-to-mass ratio of DNA is constant. Thus, for linear duplex DNA molecules, electrophoretic mobility through the agarose matrix is inversely proportional to size. For circular plasmid DNA, mobility is also based on differences in shape. In a single dimension and in the absence of a DNA intercalating agent, supercoiled plasmid DNA is more compact and therefore, has a higher mobility than relaxed DNA ((Bjornsti and Osheroff, 1999); in particular, chapter 2 by Bjornsti and Megonical). In agarose gels, DNA molecules of the same size, but with different linking number (Lk), they are resolved on the basis of differences in writhe (Wr). Because the Lk for a given closed circular DNA molecule must be an integral number, adjacent topoisomer bands differ by a Lk of one. When intact plasmid DNA molecules are relaxed, a ladder of DNA topoisomers is resolved, as shown in Figure S1. Energetically, there is little difference between DNA molecules with a similar Lk. Thus, a Boltzmann population is produced that describes a Gaussian curve. The same holds true for negatively and positively supercoiled DNAs. However, the plectonemic folding of these molecules is so compact, they will typically run as a single band in one dimension.

The addition of a DNA intercalating agent will also affect the shape of closed circular DNA molecules and therefore, their mobility in agarose gels. As discussed above, intercalator binding does not change Lk but rather induces a relative increase in Wr. As in the case of sedimentation analyses, increasing ethidium bromide or chloroquine binding first induces a decrease in the mobility of a negatively supercoiled DNA, followed by increased mobility as the plasmids DNA accumulate positive Wr. This effect has been exploited in two-dimensional gel electrophoresis to resolve a much broader range of plasmid DNA Lk. Electrophoresis in the first dimension with no or low concentrations of intercalator is followed by a second dimension, where the gel is physically rotated 90˚ and run in the presence of increased intercalator concentrations. The differences in plasmid DNA Wr, in the two dimensions, results in the resolution of an extremely broad range of DNA Lk, from negatively to positively supercoiled (Figure 3A, main text). Overall, this leads to an arc-shaped pattern consisting of discrete spots, where each topoisomer spot differs from the adjacent topoisomer by a Lk of one.

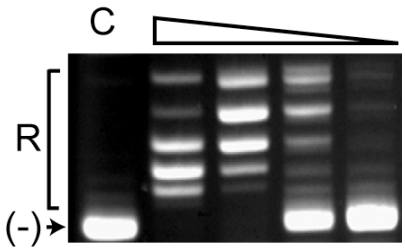

Figure S1. *Negatively supercoiled plasmid DNA was incubated with serial 10-fold dilutions of purified human wild-type TopIB topoisomerase at 37° C for 30 minutes in standard reaction conditions. The reaction products (negatively supercoiled and relaxed topoisomers) were resolved in a one-dimensional agarose gel, which was subsequently stained with ethidium bromide, exposed to UV light and photographed. Lane C contains DNA alone without TopIB.*

Supplemental Information II. Sedimentation analysis of circular chromosomes

Another method to assess the global supercoiling of large circular DNA molecules, such as the *E. coli* chromosome, is to measure changes in sedimentation induced by increasing concentrations of ethidium bromide. The underlying premise is that the Lk of a closed DNA circle cannot be altered unless one or both DNA strands are cleaved and religated. However, the binding of an intercalating agent, such as ethidium bromide, induces the unwinding of the DNA helix (i.e., a decrease in twist or Tw). Since Lk does not change, the decrease in Tw is compensated by a proportional increase in Wr. Like plasmid DNAs, the circular *E. coli* chromosome is slightly underwound. The resulting torsional strain in the DNA induces the formation of negative supercoils or plectonemes, such that the resulting DNA is very compact. Increasing amounts of bound ethidium bromide will first increase the overall dimensions of the DNA as the negative supercoils are relaxed. However, with more ethidium bromide binding, the DNA will eventually become positively supercoiled and therefore, more compact once again. The resulting changes in sedimentation coefficients can be used to accurately quantify the superhelical density of the bacterial chromosome under a variety of environmental conditions. Indeed, this approach first demonstrated the plectonemic conformation of the negatively supercoiled *E. coli* chromosome (Worcel and Burgi, 1972).

Supplemental Information III. Electron microscopic evaluation of DNA topology and replication intermediates

Some of the initial observations that closed circular duplex DNA molecules isolated from bacteria, eukaryotes, or viruses were negatively supercoiled

came from ultracentrifugation, sedimentation analysis, and electron microscopic (EM) examination of purified DNAs (e.g., (Vinograd et al., 1965)). Although EM involves the visual inspection of single DNA molecules, the DNAs themselves are isolated from cells, viral particles or in vitro reactions, and, as such, they represent the products of *in vivo* or *in vitro* bulk experiments. The subsequent analysis of the topological state of these static products is then used to infer reaction mechanism.

For example, the sedimentation analysis and EM of replicating circular SV40 DNA molecules demonstrated the accumulation of positive DNA supercoils in front of the replication fork (Sebring et al., 1971). Recent studies of more homogeneous populations of replication intermediates demonstrated the accumulation of positive plectonemic supercoils in front of the fork and positive precatenanes in the replicated DNA behind the forks (Peter et al., 1998). This direct visualization of replication intermediates confirmed the generation of positive supercoils by the movement of the replication machinery along duplex DNA. In experiments in which a chimeric T7 RNA polymerase was tethered to a GAL4 DNA binding site, EM demonstrated the accumulation of negative supercoils in the linear DNA segment between the tether site and the transcribing RNA polymerase (Ostrander et al., 1990). Thus, transcription generated a local domain of negative supercoils. Both of these studies were elegant demonstrations of DNA supercoiling induced by replication or transcription. Nevertheless, the preparation of samples for EM precludes any assessment of the dynamic nature of these processes.

Supplemental Information IV. Resolution limits of magnetic tweezers

We discuss the degrees of spatial and temporal resolution currently achievable in the case of magnetic tweezers. For a full discussion of the resolution of optical tweezers, we refer the reader to (Moffitt et al., 2008). As described in the main text, one can precisely control the topological state of the DNA molecule using magnetic tweezers to a degree determined by the directional control of the applied magnetic field, which can be very accurately controlled. However, actual measurements of the equilibrium position of the bead and thus, of the topological state of the DNA molecule, is fundamentally noisy, a consequence of the Brownian motion of the bead. Indeed, a micron-sized particle that is surrounded by fluid chaotically jiggles around in space due to the incessant bombardment of the surrounding water molecules. This is also the case with a magnetic bead attached to a surface through a DNA molecule. However, unlike free particles, the magnetic bead cannot wander too much outside its equilibrium position because the DNA exerts a force on the bead that restores it to its equilibrium position. The more the bead deviates from its equilibrium position, the more force the DNA exerts to bring it back. Indeed, defining the direction of upward and downward motion of the bead as the *z*-direction, one can describe this bead-DNA system as a spring with a spring constant, k_z. As the bead's position fluctuates about in space, the resulting spatial distribution is described by a Gaussian distribution, characterized by a mean and a standard deviation.

The spatial resolution of the magnetic tweezers is thus related to the accuracy with which one can determine the equilibrium position of this Gaussian, and the temporal resolution is determined by the time period in which this can be

accomplished. In general, more measurements of a Gaussian distribution decreases the standard error of the mean (SEM), defined as the precision of the estimation of the mean according to $SEM = \sigma / \sqrt{N}$, where s is the standard deviation of the Gaussian distribution and *N* is the number of samples taken of the distribution. However, to capture fast enzymatic events, one could decrease the time required to sample many data points (i.e., increasing the sampling frequency). However, the bead jiggles about at a characteristic frequency that is roughly between 0.1 and 100 Hz, depending on conditions (as noted above, these values are specifically valid for the magnetic tweezers technique). When studying enzymes such as topoisomerases, one works in a regime in which the characteristic frequencies are roughly between 0.1 and 1 Hz. Practically, this means that measuring very fast (e.g., 100 kHz) for a short period of time (e.g., 0.1 s) yields many measurements of the position but nonetheless, an incomplete sampling of the distribution, as the bead did not have sufficient time to explore its spatial distribution faithfully. This approach yields a skewed and incorrect estimate of the equilibrium bead position. Instead, one has to measure 'long enough,' where this time is determined by the characteristic frequency of the system. This frequency determined by parameters such as the force one applies to the DNA, the length of the DNA molecule, the viscosity of the fluid, the temperature, and the radius of the bead. Once one has measured for a long enough time to obtain a meaningful estimate of the mean, measuring longer will increase the accuracy of the measurement.

To carry out measurements on DNA molecules that can incorporate numerous supercoils, we typically work with relatively long DNA (i.e., about 7 μm or 23 kbp) and a bead that is 0.5 μm in radius that are immersed in buffer at room temperature (e.g., Figure 3C and Figure 4D, main text). For these bead-DNA parameters employed in the magnetic tweezers, we plot the standard error of the mean as a function of force for a variety of averaging times (Figure S2). In addition to reporting the SEM in units of length (nanometers), we also report the resolution expressed in number of supercoils. For example, at a stretching force of 1 pN, one needs to average the signal 1 s to obtain a resolution of one-tenth of a supercoil. It is apparent from Figure S2 that the resolution improves as a function of two parameters, the force at which the tweezers pull at the DNA and the time one takes to average. Note that the resolution in terms of supercoils does not improve as much with increasing force as the resolution in units of nm does, which reflects the fact that the change in bead height due to the addition of a plectonemic supercoil decreases with increasing force. Clearly, for optical tweezers, a similar trade-off needs to be made in that spatial resolution comes at the expense of temporal resolution. A combination of spatial and temporal resolution that is currently achievable is the detection of a 3.4 Å step at 0.5 to 1 s per step (Abbondanzieri et al., 2005).

From Figure S2, we observe that the spatial resolution of magnetic tweezers is quite good, as changes at the level of a fraction of a supercoil can be detected but that increased spatial resolution comes at the expense of decreased temporal resolution. In all experiments, a trade-off must be made. A system consisting of a shorter DNA molecule will exhibit a higher

characteristic frequency, allowing one to obtain the same spatial resolution at improved temporal resolution or improved spatial resolution at the same temporal resolution. These improvements come, however, at the expense of a decrease in the topoisomerase substrate. The choice of molecule must therefore be optimized for each experimental aim.

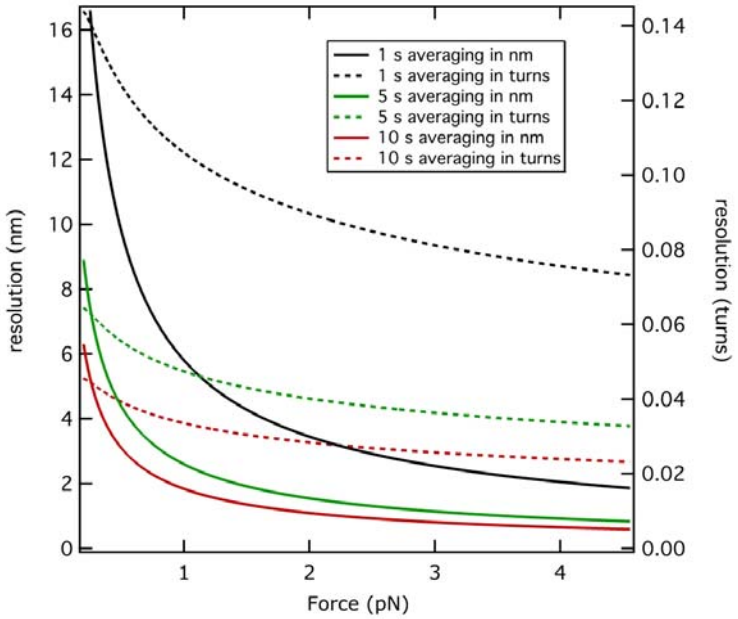

Figure S2. *The spatial resolution of the magnetic tweezers setup for a DNA molecule with a length of 7 µm and a magnetic bead with a radius of 0.5 µm. The resolution is expressed both in terms of distance and in terms of plectonemic supercoils. Additionally, these spatial resolutions are plotted for three different average times (1 s, 5 s, and 10 s averaging).*

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