Theory of Forced DNA Unzipping

Kim Ø. Rasmussen, T-11; Nikolaos Voulgarakis, CNLS; and Alan R. Bishop, T-DO

Genetic information in cells is stored in double-helical double-stranded DNA molecules. The hydrogen bonded and stacked bases are well protected in the interior of the double-stranded molecule. However, separation of the two strands is a key part of both the "reading" of DNA sequences (transcription) and DNA replication. During transcription, a transient "bubble" of single-stranded DNA is formed, to allow the enzyme that makes an RNA copy of the DNA sequence to access the DNA bases. During replication, the two DNA strands are permanently separated, to serve as templates for the synthesis of new strands.

In biochemistry experiments, DNA double-strands are routinely converted to separated single-strands by "melting" at elevated temperature (~80 °C). This approach uses thermal fluctuations to simply overwhelm the base pairing and base stacking interactions that stabilize the double helix at room temperature. In a cell, at physiological temperatures, these thermal fluctuations may still locally melt the double strand to produce small (< 30 base pairs) single-stranded regions, and it has been argued that such thermally induced bubbles play a role in at least the initiation of transcription. However, another possibility, within a cell, is that DNA strands are separated by the application of force, or in chemical terms, by enzymes whose interactions with DNA make strand separation thermodynamically favorable at ambient temperature. Therefore, it is of basic biophysical interest to analyze the separation of DNA strands by force, and recent micromanipulation experiments are beginning to accomplish precisely this feat.

It has been shown that the two strands of double-stranded DNA can be separated (unzipped) by the application of ~15 pN force applied at room temperature. Fluctuations of this unzipping force about this mean have been thought to provide a means to perhaps determine the DNA sequence: slightly higher forces were shown to correspond to DNA regions with higher *GC* densities. This result was in accord with the fact that *GC*-rich sequences have stronger base-pairing interactions than *AT*-rich sequences, causing them to thermally melt at higher temperatures, and provides further strong motivation for the experiment.

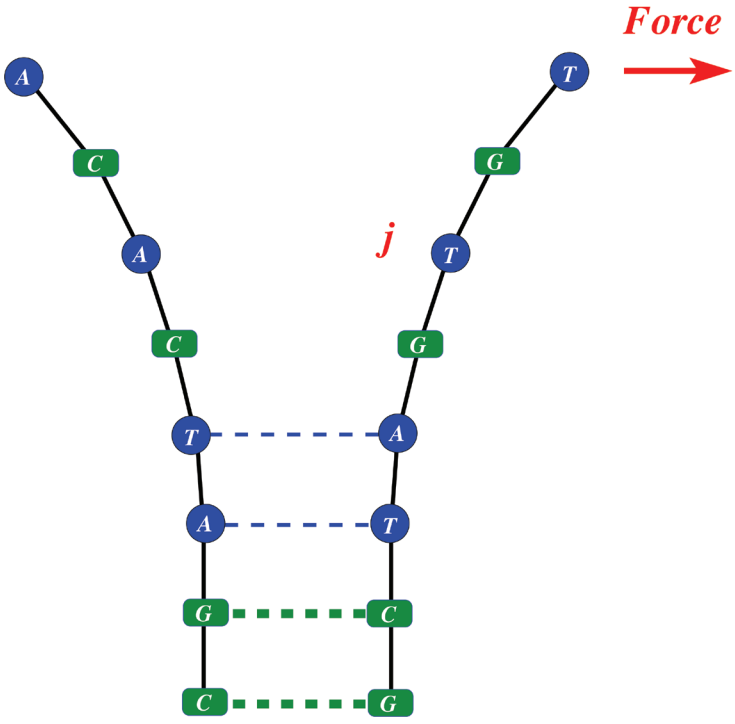

Fig. 1. Schematic illustration of forced unzipping of a DNA double-strand.

Figure 1 provides a simple illustration of a typical setup for unzipping experiments: one of the terminal base-pairs of the double-stranded

molecule is tethered to a surface (left) while the force is applied to the complementary base pair (right) by an atomic force microscope cantilever or by means of optical tweezers. The molecule is then sequentially unzipped into two single-stranded molecules, as shown. The experimental results acquired in this fashion, although impressive, are highly nontrivial to interpret and require some confrontation with modeling. Our model of choice is the Peyrard-Bishop-Dauxois (PBD) model, which is a phenomenological model originally constructed to model the thermal melting of double-stranded DNA. Lately we have been using this model also to investigate sequence dependence of the double-strand's ability to form thermally induced bubbles. We have found that certain subsequences of viral and human DNA are significantly more prone to allowing large bubbles to form. These subsequences have been experimentally confirmed to reside at transcription initiation sites. Since the PBD model has proven so effective for correctly describing double-stranded DNA bubbles, and since such bubbles are thought to play a role in the interpretation of the data acquired through unzipping experiments, we are investigating its predictions regarding forced unzipping. The main result we have obtained from this investigation is given in Fig. 2, which shows the phase diagram determining the force required to unzip the molecule at a given temperature. It is worth noticing that at room temperature the model reproduces the 15 pN, which is the experimentally required force. Recent experimental attempts have been made to determine this phase diagram, and our results are in qualitative agreement with those experiments. Most importantly we have confirmed that the PBD model is accurately describing the unzipping experiments as well as thermal denaturation.

For more information contact Kim Rasmussen at kor@lanl.gov.

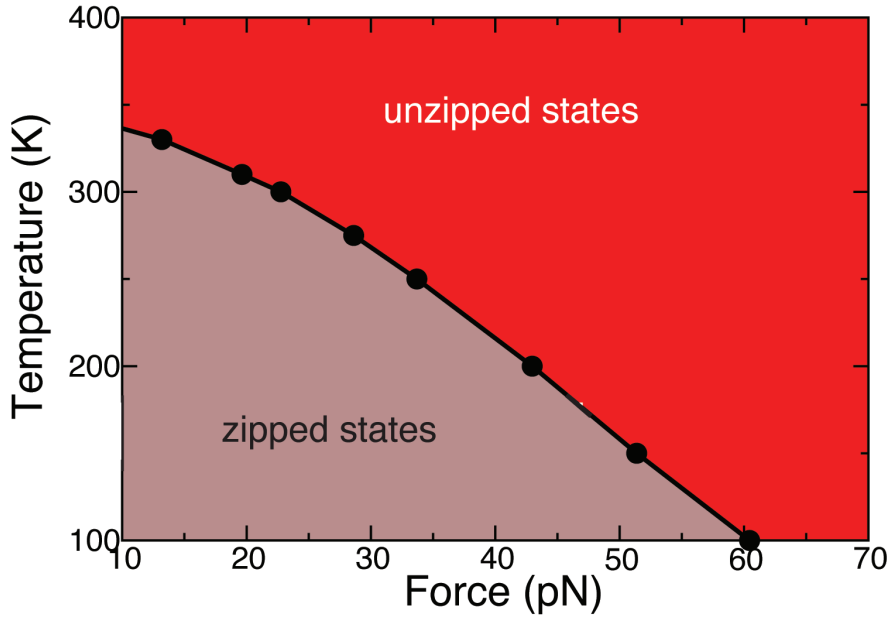

Fig. 2.
Phase diagram showing the force required to unzip the double-stranded molecule vs temperature.