COMMENTARY

Watching DNA breath one molecule at a time

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As the carrier of genetic information, DNA is the template and substrate in many fundamental biological reactions, including replication, transcription, DNA repair, recombination, and remodeling. It is now well accepted that the power of DNA in controlling gene expression is not limited to the actual genetic codes contained in DNA sequence. Compared with the well-documented chemical modifications of DNA and their functions in shaping gene expression patterns (1–3), the impact of physical properties of DNA is much less characterized, partially because they are relatively difficult to describe and measure directly. Nevertheless, it is now more and more appreciated that physical properties, such as DNA bending (4, 5) and DNA breathing (6–8), play important roles in gene expression by affecting binding and assembly of molecular machineries on DNA. In PNAS, Phelps et al. introduce to us a unique single-molecule approach to study DNA breathing dynamics and investigate the role of DNA breathing in protein assembly on DNA (9).

DNA "breathing" (or "fraying") refers to spontaneous local conformational fluctuations within double-stranded DNA (dsDNA). These conformational fluctuations lead to the breaking of base pairs at a temperature below the DNA melting temperature and exposure of these bases to the surrounding solvent. The opening of dsDNA results in a transient single-stranded DNA region (ssDNA; DNA bubble) containing one or more bases, possibly allowing proteins to gain their initial access to DNA. Several ensemble averaged methods have been used to study DNA breathing, including proton–tritium exchange (10) and proton–deuterium exchange by NMR (11), photoinduced DNA modification (12), and a combination of fluorescence quenching with fluorescence correlation spectroscopy (13). However, experimental observation of DNA breathing in real time is difficult in ensemble measurements due to the low frequency and short duration of base pair opening. Phelps et al. develop a unique approach of combining single-molecule FRET (smFRET) and single-molecule fluorescence linear dichroism (smFLD) to detect DNA breathing dynamics. The smFRET signal between a donor and an acceptor fluorophore on the same base pair of the opposite strands of DNA is detected using a total internal reflection fluorescence setup. In addition, rigid incorporation of the fluorophores and orientation constraints on the surface-tethered DNA allow the detection of the smFLD signal on polarized laser excitation, which reports on the changes in the orientation of the donor-labeled component in the laboratory frame independent of FRET. Kinetic information on a millisecond time scale is directly obtained from smFRET and smFLD time trajectories, whereas kinetic information with tens of microseconds time resolution can be extracted from analysis with a time correlation function (TCF).

As a proof-of-principle experiment, Phelps et al. apply this approach to monitor dsDNA unwinding by a T4 helicase–primase complex. During T4 bacteriophage replication, the helicase–primase complex binds at the dsDNA/ssDNA fork junction and processively unwinds the dsDNA for DNA synthesis (14). DNA unwinding is tracked by the drop in the FRET signal, and at the same time, the FLD signal from the donor changes from a relatively constant nonzero value, indicating that dsDNA is rigidly oriented relative to its surface tether point, to a broadly fluctuating signal around 0, suggestive of an entirely free rotation of the resulting ssDNA. Strikingly, the FLD signal shows highly dynamic changes even when the FRET signal shows a constant value, highlighting the power of monitoring multiple observables. As illustrated in these experiments, the smFLD measurement complements smFRET detection in two ways: first, it can report on conformational dynamics

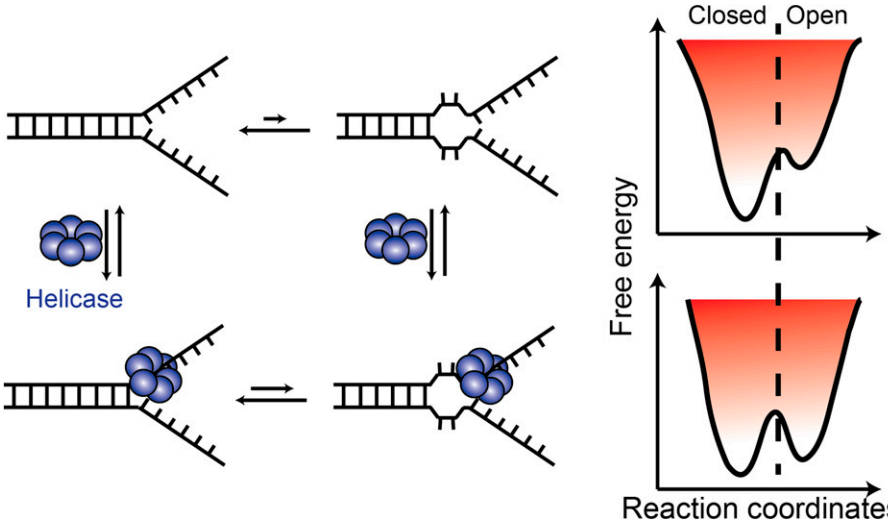

Fig. 1. Helicase takes the advantage of intrinsic DNA breathing to be preferentially loaded at the fork junction, and binding of helicase in turn reshapes the energy landscape of DNA breathing dynamics.

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that the FRET signal is blind to, and second, any ambiguity between a real conformational change of DNA and photophysical change of the fluorophores can be addressed by measuring smFLD and smFRET simultaneously.

The decay rate of TCF reflects DNA breathing dynamics and the closing rate of the DNA bubble. In the absence of any protein, opening of DNA is very transient, with an average lifetime of the bubble on the order of ∼100 μs, consistent with previous ensemble measurement (13). Compared with the interior region of the dsDNA, the closing of the bubble is slower near the fork junction, reinforcing the previous observation that DNA breathing dynamics is location dependent and suggesting that the helicase may take advantage of DNA breathing dynamics to be preferentially loaded at the fork junction (7).

How does binding of helicase in turn influence DNA breathing? Phelps et al. further introduce T4 helicase to the DNA construct, in which the donor and acceptor fluorophores are attached near the fork junction. With nonhydrolyzable GTP analog GTPγS, but in the absence of primase, T4 helicase weakly binds to the fork junction (7, 15). Helicase binding dramatically increases the average magnitude of DNA breathing fluctuation and the lifetime of the open conformation. These results clearly demonstrate that the thermodynamic equilibrium of DNA breathing can be tuned by binding of a helicase (Fig. 1). Based on these observations, the authors propose a binding mechanism of T4 helicase–primase, which involves cooperative interplay between intrinsic DNA conformational dynamics and helicase binding: helicase uses the spontaneously assessed open conformation near the fork junction and in turn stabilizes this open conformation for subsequent recruitment of primase.

In addition to its role in the assembly of the helicase–primase complex as proposed in this study, DNA breathing can facilitate other processes, for example, by regulating transcription factor binding (8). More systematic studies in the future may unveil how the location and kinetics of DNA breathing are correlated with the interaction with many other DNA binding proteins. The work presented by Phelps et al. provides us with a very powerful single-molecule approach to investigate the intrinsic DNA conformational fluctuation. It can be foreseen that this approach can potentially be used in genomewide analysis of DNA physical properties and their contribution to an additional layer of gene expression regulation.

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