On the common pathways of deformation: RNA vs DNA under interrogation

DNA/RNA molecular structures are highly susceptible to environmental stimuli and consequent deformations significantly impact on genetic regulation and association with proteins for various functions (1, 2). As physiological processes are complicated and hard to be monitored directly, efforts have been made in well controlled experimental conditions on measuring DNA/RNA conformational responses to representative stimuli: from earlier applying mechanical tensions on DNA to monitor twist-tension or twist-stretch coupling (3, 4), to later probing temperature dependence of DNA persistence length or twist (5, 6), and to more recently imposing salt concentration variation to induce double-stranded (ds) RNA conformation change (7) or DNA twist (8, 9). Meanwhile, comparative studies had been conducted between dsRNA and DNA, revealing comparable mechanical responses but notable distinctions (10-12). In PNAS, Tian et al. present a work demonstrating particularly dsRNA deformations upon salt and temperature changes, and suggesting a common pathway of the RNA deformations via helical twist-groove coupling, independent of stimuli including salt, temperature, and stretching force (13). The RNA deformations have also been compared with DNA deformations studied recently (9), in which an alternative common pathway via helical twist-diameter coupling reveals, irrespective of the types of stimuli as well.

RNA/DNA twisting using magnetic tweezers (MT)

To monitor the twist change of dsRNA/DNA, magnetic tweezer (MT) as a primary experimental tool has been well employed. In an MT apparatus, magnetic beads are manipulated via forces in the presence of an external magnetic field (14). Early implementation of MT was initiated on measuring structural mechanical responses of single molecule DNA (15-17). The coupling between DNA twisting and stretching degrees of freedom were then particularly investigated, e.g., using rotor bead tracking technique along with applying tension to single molecule DNA via MT (3). It was found that below a critical tension of ~ 30 pN, DNA overwinds as well as lengthens when it is stretched. Another work using MT methods quantitatively determined that the extension of a stretched DNA molecule increases by ~ 0.42 nm for every excess turn applied to the double helix, and the measurements were justified via modeling torsionally restrained DNA subject to energy minimization (4). Using similar MT techniques, dsRNA molecule was studied later, i.e., upon solving RNA assembly issue during implementation of single-molecule external forces and torques. It was shown that beyond similarities with DNA on melting and conformational transitions etc., dsRNA shortens upon overwinding or twisting (10), in contrast with lengthening of DNA under similar conditions. In PNAS, rather than applying mechanical force, Tian et al. (13) have imposed salt and temperature changes, and demonstrated notably that the coupling between helical twist and length degrees persists and dictates the dsRNA deformations, in general.

Combining MT experiments with molecular dynamics (MD) simulations and theoretical analyses

In current implementation, to determine RNA twist change in the MT experiments, one first counts the rotation number corresponding to a maximum RNA extension upon the RNA relaxation at a certain salt/temperature condition (see **Figure 1** middle), and then monitors the change of the maximum RNA extension induced by variation of the condition, i.e., due to salt ionic strength or temperature change. The experimental results show that the twist of dsRNA increases as the salt concentration increases. Meanwhile, the RNA twist decreases as the temperature increases. Instead of manipulating force or torque experimentally, Tian et al. further employed molecular modeling and simulation techniques to probe

which internal degrees of freedom are coupled and how, focusing on the RNA deformations of twisting induced by salt and temperature change.

All-atom molecular dynamics (MD) simulations apply classical mechanics to biomolecular systems to monitor conformational changes (18-20), on top of molecular force field developments (21-23). In PNAS, Tian et al. employed the atomic MD simulations to reproduce the salt-induced RNA twisting first, semi-quantitatively (13). The simulations were conducted for a construct of 25-bp dsRNA with explicit water solvent at different concentrations of NaCl, KCl, and RbCl, at room temperature and then higher (~20 to 35 C°). Implementing typical force field (with adjustment for Na^+) and simulation package, hundreds of nanoseconds atomic MD simulations were conducted for each system, and the simulated RNA conformational changes were analyzed. The analyses suggest that upon the salt concentration or solvent ionic screening increase, electrostatic repulsion between RNA strands weakens, hence the RNA major groove size decreases, which is transduced to increase RNA twist via the twist-groove coupling. To illustrate the coupling, the authors constructed a two-dimensional potential of mean force (PMF) from the simulations via sampling the RNA helical twist and groove size degrees, revealing negative coupling between the two, i.e., the larger the twist, the smaller the groove size, and vice versa. With conformational coupling constants derived from the MD simulations, further theoretical analyses were conducted to fit with experimental data, based on the idea that an effective force is generated on the two RNA strands, i.e., dominated by the phosphate-phosphate electrostatic repulsion. Upon changes of salt concentration or species, which modulate effectively the Debye screening length to affect the intramolecular electrostatic repulsion and cation binding to the dsRNA major groove, the force induces changes on the RNA groove size and then the helical twist. In case that temperature rises, one expects another effective force due to conformational entropic effect, i.e., to enlarge the RNA groove size. Such an entropic force can be estimated by segregating the energetic or enthalpic part from the temperature-dependent or entropic part of the PMF obtained from the MD simulation. As a result, the entropic force that enlarges the groove size with increasing temperature then reduces the RNA twist, again, via the negative twist-groove coupling. As such, one sees how the RNA twist couples with the major groove size upon the salt or temperature impact. Nevertheless, one may still wonder why dsRNA does not behave as DNA, i.e., to deform via the twist-diameter coupling.

Distinction between the common pathways of RNA twist-groove and DNA twist-diameter coupling

To further illustrate the mechanism behind the RNA helical twist-groove size coupling, additional degrees of freedom of the RNA structure were explored in the simulations. It was found that the diameter of the dsRNA helical structure hardly changes upon the variation of salt or temperature (13), in contrast with the finding on diameter variations in the DNA deformations (9). One can envision that as the helical strand of RNA keeps a fixed contour length, the diameter size of the helical structure remains constant. In such a case, if one overtwists the RNA structure, the helical pitch (i.e., height of one turn) has to shrink, which leads to a reduced groove size. The mechanism was noticed previously via MD simulation studies on mechanical responses of dsRNA and DNA under constant stretching force (12) (see **Figure 1A**). It was suggested that the extra oxygen of RNA with respect to DNA impedes the RNA diameter variation, which turns about to be a necessary condition to support the RNA twist-groove coupling. In comparison, the diameter of the DNA helical structure is more susceptible to change than the groove size, so the twist-diameter coupling dominates the DNA deformations (9). In order to explain measurements from twist-stretch experiments (10), an earlier MD simulation study also suggested that overwinding RNA results in

more compact conformation with a narrower major groove, while overwinding DNA results in a reduced helical radius (11). Above all, it is in PNAS, Tian et al. suggest that 'universality' of the dsRNA deformations applies, on top of combined experimental and computational studies evidencing twist-groove coupling in RNA induced by salt and temperature changes, while incorporating previous findings on the mechanical stimulus into current framework. Putting all together, the universality in regard to common deformation pathways of dsRNA and DNA have been highlighted, respectively, via two well collapsed lines of twist-groove and twist-diameter coupling, under salt, temperature, and force stimuli (**Figure 1B** middle). In addition, Tian et al. thoroughly recorded how various internal degrees of dsRNA change, e.g., on the helical bend (roll) and tilt, in addition to twist, groove, and diameter changes, from the MD simulations.

Implications for protein binding to RNA and DNA

As the mechanisms behind the universality rely on the double helical geometry and RNA/DNA internal rigidities, for protein-RNA/DNA interactions that can be regarded as perturbations to the helical geometry and elasticity, one would expect that RNA/DNA naturally reacts by displaying the common pathway of deformations, so that to lower the energy cost to facilitate the protein binding. Follow this idea, Tian et al. additionally tested a handful of dsRNA/DNA binding proteins, to examine the corresponding RNA/DNA deformations upon protein association (13). Six protein-RNA/DNA complexes with high-resolution structures taken from protein data bank were simulated via atomic MD and further analyzed, in comparison with the standalone dsRNA or DNA structures. The obtained RNA/DNA deformations do show some extent of correlations between twist and groove/diameter size, in particular, when changes of individual degrees are normalized, i.e., to reduce RNA/DNA sequence impacts. Anyhow, such expectations on the protein-RNA/DNA association are based on the assumption that the protein impacts act as perturbations to the dsRNA/DNA helical structures. In case that the double helical structures of the RNA/DNA are to be substantially altered for certain physiological functions, or upon extra energetic supply, the deformations may go beyond the currently suggested common pathways or universality.

Legend

Figure 1. (A) Previous computational work (12) suggested mechanical responses of dsRNA and DNA (images adapted from (12)). (B) In PNAS, Tian et al. used MT for monitoring RNA peak extension change to determine the RNA twist change (*left*) (13), and suggested the universality of dsRNA and DNA deformations upon salt, temperature, and force changes (*middle*). The structural schematics of dsRNA and DNA are shown for the twist-groove and twist-diameter coupling (9), respectively (images adapted from (13)).

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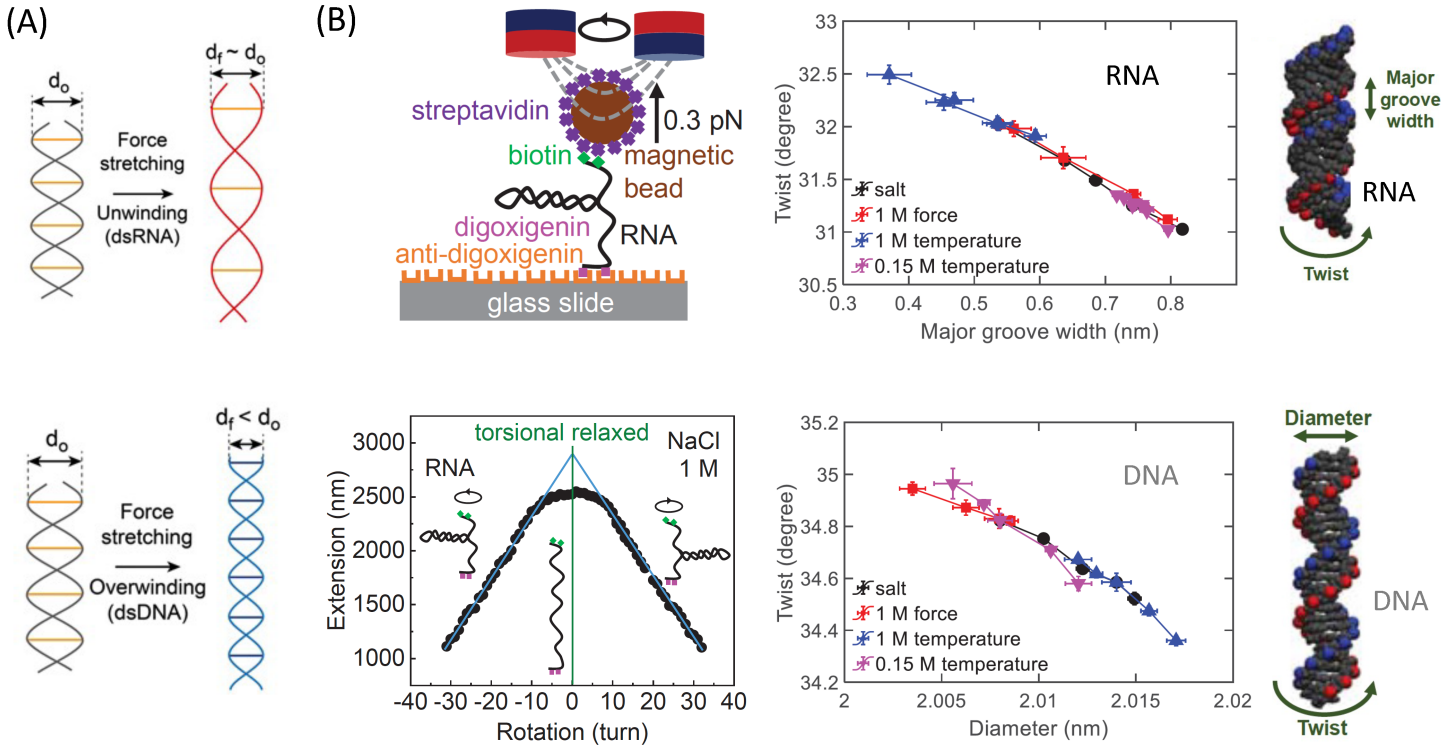
(A)
d_o
$d_f \sim d_o$
Force stretching
Unwinding (dsRNA)
d_o
$d_f < d_o$
Force stretching
Overwinding (dsDNA)
(B)
streptavidin
biotin
magnetic bead
0.3 pN
digoxigenin
anti-digoxigenin
RNA
glass slide
torsional relaxed
NaCl 1 M
RNA
Extension (nm)
Rotation (turn)
Twist (degree)
Major groove width (nm)
RNA
salt
1 M force
1 M temperature
0.15 M temperature
Major groove width
RNA
Twist
DNA
Diameter (nm)
Diameter
DNA
Twist