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DNA acting like RNA

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Abstract

Over the last decade or so, secondary non-B-DNA structures such as G-quadruplexes and i-motifs have come into focus as biologically functioning moieties that are potentially involved in telomeric interactions and the control of gene expression. In the present short review, we first describe the structural and dynamic parallels with complex RNA structures, including the importance of sequence and ions in folding, and then we describe the biological consequences of the folded structures. We conclude that there are considerable parallels between secondary and tertiary structures in RNA and DNA from both the folding and the biological perspectives.

Keywords

G-quadruplex; i-motif; RNA; secondary DNA structure

Introduction

Over half a century ago, the structure of DNA was elucidated through the work of Franklin, Watson and Crick [1,2]. For many years after the discovery of duplex DNA, it was widely accepted that it only existed in the right-handed B-form of the molecule. However, sustained interest in the physical chemical properties of the molecule and advances in molecular assays have shown that this is not the case. DNA is capable of assuming a wide variety of helical and non-helical topoisomers contingent on sequence, chemical modification and molecular torque [3–5]. Atypical non-B-form helical DNA topoisomers, such as H- (triplex), Z- (left handed) and A-DNA, have been shown to arise from a variety of experimental conditions [3,6,7]. Furthermore, DNA can adopt alternate topoisomers when one of its strands loops back on itself, as seen with hairpins, triplexes, G-quadruplexes and i-motifs [8–11] (Figure 1). Although the biological roles and relevance of these structures are still a current topic of debate, the formation of some of these structures has been shown in biologically relevant systems [12]. Interestingly, the dynamics of the less stable and nearly identical daughter molecule, RNA, parallel many of those observed in DNA [13–15]. For the purposes of the present review, we draw the similarities between higher-order DNA and RNA secondary structures, describing how their folding dynamics, higher-order secondary structures and biological functions are more alike than generally appreciated.

Conditions that give rise to secondary DNA structures in eukaryotic cells

Under physiological conditions, the transition from B-DNA to the less energetically favourable topoisomers is often driven by the torsional stresses generated by the RNAP (RNA polymerase) machinery [6,16,17]. As the RNAP machinery transcribes a nascent strand of RNA, it is capable of two means of travel: (i) winding around the helical DNA, or (ii) the DNA is pulled through the RNAP as the sequence is transcribed. If one considers the size of the multi-subunit RNAP machinery, and the growing strand of RNA, it becomes clear that winding around the template DNA is both sterically and energetically unreasonable [4,6,16,18,19]. It is more energetically favourable for RNAP to pass the template DNA through its multi-subunit complex [19,20]. As the RNAP processively transcribes the DNA into RNA, it generates a local negative and positive supercoiling upstream and downstream of the complex respectively [4,16,18–20]. Positively and negatively supercoiled DNA are torsionally strained, and the energy associated with these stresses can manifest in the form of twist and writhe. Although DNA topoisomerases have been known to alleviate these strains, they are generally low in abundance [18,21–23]. It is therefore unlikely that they respond immediately to all regions of positive/negative superhelicity in an actively transcribed genome. Consequently, a local melting of duplex DNA is associated with transcriptionally active areas of the genome [4,16] (Figure 2). These locally unwound single-stranded regions are capable of adopting DNA secondary structures to relieve torsional stress [4,12,16,22,24,25].

Sequence-dependence of formation of G-quadruplexes

Whereas superhelicity generated from the RNAP has been shown to generate favourable conditions for the formation of secondary structures, such as cruciform DNA and G-quadruplexes, this alone is not sufficient for their formation. All DNA secondary structures require specific sequence contexts for formation, just as the sequence of a promoter encodes the binding sites of specific proteins and enzymes [10,11,26]. For example, cruciform structures can arise from palindromic sequences, while G-quadruplex structures can form from at least four contiguous runs of two or more consecutive guanines [11]. As a basis for comparison of the dynamics of DNA and RNA throughout the present review, we focus on G-quadruplex structures, although many of the same concepts may also be true for i-motifs. These G-quadruplexes form through the Hoogsteen base-pairing of four guanines that nucleate around a univalent cation (sodium or potassium) [11]. This forms the fundamental building block of a G-quadruplex known as a G-tetrad (Figure 3). The planar nature of the G-tetrad permits stacking, and this is stabilized further by co-ordination with K^+ or Na^+ between the tetrads. In promoter elements, most, but not all, of the G-quadruplexes arise from four contiguous runs of guanine to form an intramolecular structure.

G-quadruplex variety and dynamics

G-quadruplexes have been shown to form a variety of topologies, including parallel, antiparallel, mixed parallel/antiparallel, heptad-tetrad, kinked and snapback motifs [11,24,25,27–29] (Figure 4). Strand directionality and varying loop length allow for unique G-quadruplex structures to accumulate and, in some cases, to interact, forming higher-order tertiary structures [27,28]. The most complex promoter DNA structure so far examined involving G-quadruplex secondary-structure interactions is encoded within the *hTERT* (human telomerase reverse transcriptase) promoter [28]. The 12 runs of guanines within the *hTERT* promoter fold into a parallel G-quadruplex, separated by a linker of seven bases, from a mixed parallel/antiparallel G-quadruplex that contains a 26-bp loop (Figure 4, middle circle). Folding in this manner sequesters all three Sp1 (specificity protein 1)-binding sites in this element to silence gene expression. Although the specific thermodynamic interactions

remain to be elucidated, this suggests an interaction between the two G-quadruplex secondary structures to form a tertiary complex [28].

DNA acting like RNA

As a starting point to understanding DNA folding dynamics, it is important to consider the torsional strain induced by the transcription machinery acting as the driving force for the interconversion between duplex DNA and its various topologies (see above). At this point, the similarities between DNA- and RNA-folding dynamics become markedly evident. Perhaps the most intuitive and most important similarity between the two is their sequence composition. There are four planar aromatic purine/pyrimidine bases attached to a sugar phosphate backbone in which the deoxyribose may assume one of only two possible conformations. The dynamics of these building blocks in both DNA and RNA behave similarly [3,8,10–12,14,15,30,31]. The compilation of hydrophobic, Van der Waals and hydrogen-bond acceptors and donors allows for the formation of a various analogous secondary structures. The accumulation of these forces acting in various conformations results in analogous structures, such as hairpins, cruciform junctions, G-tetrads/quadruplexes, helices, loops and bulges [3,4,10,14–16,32–34].

Importance of ions in the folding of RNA and DNA

The formation of topoisomers in both systems is not solely dependent on sequence; ions play an important role in the stabilization of charge and structure. Specifically, positively charged ions such as Mg^{2+} , Na^{+} and K^{+} allow for the stabilization of DNA and RNA secondary structures [11,14,15,35–38]. Univalent cations such as K^{+} play a critical role in the stabilization of DNA and RNA G-tetrads/quadruplexes. Univalent cations interact with the phosphate groups, effectively shielding their negative charge to facilitate folding of higher-order RNA structures, an effect analogous to that of ionic strength on polyelectrolytes [15,31,35]. Bivalent cations play an important role in the stabilization of secondary and tertiary motifs in RNA [14,15,35,36]. In a recent paper, it has been suggested that the Mg^{2+} plays an important role in the formation of G-quadruplex structures [39]. As secondary motifs interact to form tertiary structures, there can be local association of hydrogen-bond acceptors to create an ion-chelating pocket. The presence of bivalent cations in solution stabilizes these tertiary structures, allowing for the formation of even higher order structures. In the absence of a mediating ion, the thermodynamically unstable tertiary structure would be short-lived. This dependence on ions is observed in the formation of RNA pseudoknots and riboswitches and with DNA runs of GC, where the Mg^{2+} ion can interact with the N^7 and carbonyl oxygen of guanine and the 4-carbonyl oxygen of cytosine [34,40–44].

In both DNA and RNA, the energies involved in secondary-structure formation are greater than those involved in tertiary structural interactions. This permits secondary structures to exist in a stable form by themselves, further allowing time for higher-order structures to accumulate. This hierarchical processing and development of higher-order RNA structures has been extensively studied and reviewed [15,24,31–36]. Over the last 30 years, the ‘RNA-folding problem’ has been the topic of an intense amount of investigation. The result has been a remarkable number of insights into the RNA sequence structure–function relationships and the emergence of various rules and algorithms for predicting RNA secondary structure [14,15,32]. Although work on the ‘DNA-folding problem’ is still in its infancy, its advances will surely be aided by the similarities/parallels with RNA. As a side note, DNA is the prototypical nucleic acid archetype; therefore, considering the age-old question of ‘which came first: the chicken or the egg?’, one could reason that a more appropriate title for this review could be RNA acting like DNA.

Structural and biological consequences of RNA and DNA folding

Sensing and gene-control functions are most commonly associated with proteins in their role as transcriptional factors. However, it is now well recognized that RNA can also play a role in both of these functions, particularly in non-coding regions of RNA, such as in 5'- and 3'-UTRs (untranslated regions). An important example here is the riboswitch contained in 5'-UTRs. These riboswitches are located in the mRNA and can bind directly to a small target molecule that is the end-product of the catabolic pathway in which the gene containing the 5'-UTR codes for an intermediate enzyme. This target molecule in turn modulates the gene expression and thus is able to regulate its own expression levels through this negative-feedback mechanism. This is illustrated with the lysine riboswitch, shown in Figure 5, in which binding of lysine stabilizes the P1 helix, which results in the formation of the terminator and suppression of expression of the downstream gene [45]. Conversely, in the absence of lysine, the anti-terminator is formed.

These sensing and gene-control functions can also be extended to DNA, particularly in genomic regions flanking the transcriptional start site, where negative supercoiling generated by transcription can induce sufficient torque to melt duplex DNA (see above). Bioinformatics studies show that putative G-quadruplex-forming regions are unusually concentrated in these regions [46], and this is conserved across mammalian species [47]. In the promoter element of *c-Myc*, there is an equilibrium between duplex, single-stranded and secondary DNA structures revealed in negatively supercoiled DNA by chemical and enzymatic footprinting [12]. These different forms of DNA act to control the on/off switch for *c-Myc* transcription from the P1 and P2 promoters, using a combination of transcriptionally induced negative supercoiling and NM23-H2 and nucleolin proteins, which either unfold or fold the *c-Myc* G-quadruplex to activate or turn off transcription (Figure 6). In the case of the *c-Myc* promoter, there is a predominant G-quadruplex and i-motif (Figure 6) that forms to sequester the duplex or single-stranded elements that would otherwise serve to bind to the activating transcriptional factors that turn on *c-Myc* transcription [24]. Thus a direct mechanosensor system is responsive to the transcriptionally induced negative supercoiling, providing part of the mechanism for gene activation. A second mechanosensor system involving a single-stranded DNA element called FUSE (far upstream element), together with two competing DNA proteins [FBP (FUSE-binding protein) and FIR (FBP-interacting repressor)], controls the rate of transcriptional firing [4,6,16].

In other G-quadruplex-containing promoter elements, the complexity is more pronounced, with multiple runs of guanines that can form alternate G-quadruplexes from overlapping sequences [11,25]. One such example is PDGFR β (platelet-derived growth factor receptor β), where four different G-quadruplexes can form within a 34-bp sequence containing seven guanine runs [48]. Intriguingly, differential drug binding to the G-quadruplexes results in different outcomes on transcription, suggesting that, in this system, the on/off and firing rates may both be controlled through this mechanosensor unit by differential display of the different G-quadruplexes that have distinct folding patterns. Although the molecules identified are not those produced naturally by cells to switch between different G-quadruplexes, this system has analogies to the riboswitches described above (see Figure 5), in which different RNA conformers are selectively bound by cellular metabolites.

Future prospects

These are still very early days in understanding the full significance of DNA secondary structures such as G-quadruplexes and i-motifs in controlling processes such as gene expression. It should be emphasized that the vast majority of systems to be examined have been carried out in *ex vivo* systems, and the existence of these structures is based on

circumstantial evidence. Only a handful of promoter G-quadruplexes have been examined (Figure 4), and, in most of these cases, with the exception of c-Myc, little is known about the proteins that manage the formation and dissipation of these structures and their biological roles. Even less is known about the possible structures and functions of the i-motifs that can originate from the C-rich strands in these same promoters [49]. Even so, it is already possible to recognize that Nature has devised a means to form these structures from duplex DNA using transcriptionally induced negative superhelicity, and the diversity and complexity of these structures [25] suggest a role in selective modulation of gene expression. In this respect, the secondary and tertiary structures of G-quadruplexes in promoters such as *hTERT* and the complexity of the G-quadruplexes in the *Bcl-2* and *PDGFR β* promoters suggest a parallel with RNA structures, such as riboswitches found in 5'-UTRs. Thus DNA is starting to look and act more like RNA, hence the title of the present short speculative review.

Abbreviations used

FUSE	far upstream element
FBP	FUSE-binding protein
hTERT	human telomerase reverse transcriptase
PDGFRβ	platelet-derived growth factor receptor β
RNAP	RNA polymerase
Sp1	specificity protein 1
UTR	untranslated region

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Figure 1. Models of DNA hairpin and triplex structures and folding patterns of the G-quadruplex and i-motif in the *c-Myc* promoter

The hairpin DNA picture was created using the NMR structure of hairpin B-DNA (PDB code 2VAI) and the triplex DNA picture was created using the NMR solution structure of DNA triplex (PDB code 1BWG). These two images were created using Sybyl 8.0 software (Tripos).

Figure 2. Mechanics of the transmission of the torsional stress through the chromatin fibre

If RNAP is moving from left to right without rotating in three dimensions, then, owing to its helical structure, the DNA must be screwed through the enzyme. The DNA behind the transcriptional machinery becomes undertwisted (negatively supercoiled). This supercoiling might diffuse into an upstream region due to the highly dynamic nature of the DNA–nucleosome core interaction and structural plasticity of chromatin fibres and must be accommodated by supercoiling in the linker region, rotation of nucleosomes *en bloc* or altering the stereochemistry of the histone octamer–DNA complex. Reproduced with permission from [24], originally published in [6].

Figure 3. Two examples of intramolecular G-quadruplexes formed in the promoters of Bcl-2 (left) and c-Myc (right) from a tetrad (centre)
 The number of bases in the loops are indicated in parentheses under each quadruplex.

Figure 4. The six hallmarks of cancer [50] shown with the associated G-quadruplexes found in the promoter regions of these genes

The various G-quadruplexes differ by folding pattern, number of tetrads, loop size and constituent bases. In the middle circle is shown the proposed model of the tandem G-quadruplex structure formed in the *hTERT* core promoter. The two G-quadruplexes formed by G-tracts 1–4 (G4-1:3:1) and G-tracts 5, 6, 11 and 12 (G4-3:26:1) are linked by the sequence C¹⁹–G²⁵. The *hTERT* promoter sequence, with the Sp1-binding sites indicated, is shown at the bottom below the proposed model. Each of the individual quadruplexes is representative of secondary structures, whereas the compilation of both is a tertiary structure. G-tracts involved in the G-quadruplex structures are shown in red and numbered [28]. Reprinted with permission from [24] and from [28]. © 2009 American Chemical Society.

Figure 5. Transcriptional attenuation mechanism in the lysine riboswitch

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Figure 6. Models of the different promoter forms within the c-Myc NHE III₁

(A) Duplex representation of the promoter without any proteins bound. (B) Binding of Sp1 to the duplex structure leading to activation of c-Myc expression. (C) Binding of hnRNP (heterogeneous nuclear ribonucleoprotein) K and CNBP (CCHC-type zinc finger nucleic acid-binding protein) to the single-stranded C- and G-rich regions respectively, leading to activation of c-Myc transcription. (D) Repression of c-Myc transcription when Sp1, hnRNP K and CNBP are not bound, leading to the formation of the G-quadruplex and i-motif. Reproduced with permission from [51].