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DNA structure and human diseases

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1. ABSTRACT

Recently, multiple lines of evidences have shown that non-B DNA structures within the human genes may lead to chromosomal translocations, deletions and amplifications in cancer and other human diseases. In this review, we summarize the current literature on non-B DNA structures, and discuss their role in genomic instability and diseases.

2. INTRODUCTION

Since the time Watson and Crick described the right-handed DNA double-helix (B-DNA), many biologists have regarded DNA as a very uniform molecule. However, many studies during the last several decades have led to the understanding that other forms of DNA structure exist. During this period, it was noted that specific defined sequences such as mirror repeats, direct repeats, homopurine-homopyrimidine tracts (including G tracts), inverted repeats can form a number of altered DNA structures (non B-DNA structures). It was noted that specific poly-A tracts can result in DNA bending. Inverted repeats can form cruciform DNA structures. Other such structures included left handed Z-DNA in alternating purine.pyrimidine sequences, triplex DNA structures at homopurine.pyrimidine tracts with mirror repeat symmetry, G-quartets (tetraplex structures) at guanine-rich sequences, and slipped structures promoted by direct repeats (1). In addition, long GAA repeats can form sticky DNA (2). Although good progress has been made in understanding the structures formed by different sequences, there is still very little known about the biological functions of such structures *in vivo*.

The recent human genome sequence analyses have shown that sequences with the potential to form non-B DNA structures are abundant in our genome. Repeat sequences seem to be the major contributors of non-B DNA structures. Although we still do not know much about the biology of these structures, recently there were many studies implicating such structures in various human diseases (3-5). The altered DNA conformations affect DNA polymerase stalling, in some cases leading to nicks and double-strand breaks in the DNA (6). In this review, we first describe the features and requirements of many known non-B DNA structures. Then we discuss examples

Table 1. Different non-B DNA conformations and their characteristics

Name of non-B DNA structure	Physical nature	Sequence requirement	Nature of hydrogen bonding	pH dependence	Requirement of cations	Implicated in disease
Z-DNA	Left handed DNA	Alternating pyrimidine-purine sequences	Watson-Crick paired	No	K^+, Na^+, Rb^+, Cs^+, Li^+, Mg_2^+	_
Cruciform DNA	Intra-molecular B-helix with a loop	Inverted repeat sequences	Watson-Crick paired	No	Na^+	+
Triplex DNA (H-DNA)	Three stranded	Homopurine Homopyrimidine sequences with mirror repeat symmetry	Hoogsteen paired	Yes, acidic or neutral pH	$Mg2^+$ Spermidine Spermine	+
Quadruplex (G-Quartet)	Four stranded	Tracts of guanine sequence	Hoogsteen paired	No	K^+, Na^+	+/-
RNA-DNA hybrid	R-loop structure	G:C repeat sequences	Watson-Crick paired	No	No	+
E-DNA (e motif)	Extrahelical DNA	Unpaired protonated cytosines	C^+C paired	Yes pH 7-8	No	-
I-DNA (i motif)	Intrahelical DNA	Protonated cytosines	C^+C paired	Yes acidic pH	No	-
Slipped DNA	Misaligned DNA	Direct repeat sequences	Watson-Crick paired	No	No	+

of some of the human diseases where the pathogenic mechanism is thought to involve a non-B DNA structure.

3. Non B-form DNA structures

Normally, the majority of DNA adopts a standard Watson-Crick paired B-form configuration. Depending on the nature of the sequences, some regions of DNA can form non-B DNA structures at least transiently (1, 7-12). Most of the time, such structures are formed during replication, recombination or transcription. The type of hydrogen bonding within the non-B DNA structures can vary from the normal Watson-Crick pairing to Hoogsteen hydrogen bonding, depending on the type of non-B DNA structure formed. In addition to the sequences involved, other factors that influence the DNA structure include supercoiling, polyvalent cations and pH.

Among the DNA structures thus far described *in vitro*, cruciform DNA, Z-DNA, triplex DNA (H-DNA), sticky DNA (a derivative of triplex DNA), tetraplexes (G-quartets), slipped structures, RNA:DNA hybrid, I-DNA (i-motif), and E DNA (e-motif) have been described and characterized (Table 1).

Hairpin and cruciform structures can be formed in inverted repeats. Each strand of the inverted repeat folds at the center of symmetry and adopts an intramolecular B-DNA helix capped by a single-stranded loop at the top. A hairpin structure is formed at one strand of an inverted repeat whereas a cruciform structure consists of two hairpin structures, both in each strand at the same position of the DNA. The loop region may vary from a few bp to several bp. The cellular processes like replication, transcription cause unwinding of the duplex, providing the single-stranded region, thereby giving the repeat sequences the opportunity to fold back and form alternative base pairs within the same DNA. Cruciform/ hairpin structure formation requires only Watson-Crick hydrogen bonding (1, 5, 12). A 14 bp palindrome is enough for hairpin structure formation in *Saccharomyces cerevisiae*, with the negative supercoiling providing the energy for formation of structure (13). Many repeat sequences associated with neurological disorders are shown to form such hairpins.

The left handed Z-DNA forms *in vitro* at regions of alternating pyrimidine-purine, (YR-YR)n sequences. The transitions from the right-handed B-form to the left-handed Z-form is accomplished by a 180^o flip of the base pair through a rotation of every other purine from the anti to the syn conformation and a corresponding change in the sugar puck from the C2'- to the C3'-end conformation. In contrast to B-DNA, Z-DNA does not have a major groove (1, 14).

Triplex DNA has been studied since 1957 *in vitro*. Triplex DNA is formed by laying a third strand into the major groove of the Watson-Crick duplex DNA (Figure 1). There are two different types of triplexes studied, intermolecular and intramolecular, depending on the kind of substrate DNA used. Intramolecular triplex DNA (H-DNA) forms within polypurine.polypyrimidine sequences (including poly-G sequences, (15)) with mirror repeat symmetry (1, 7). Both Hoogsteen and Watson-Crick pairings contribute to triplex DNA formation. Factors that influence triplex DNA formation include pH, divalent cations, and negative supercoiling (which can exist within living cells (16). There are two types of triplexes, R.R.Y triplexes and Y.R.Y triplexes. An R.R.Y triplex formation is favored at neutral pH (7.0) in the presence of cations, whereas an Y.R.Y triplex is favored at acidic pH (4.8) (1, 7, 10, 15, 17-19). It is also reported that DNA sequences that can form H-DNA can act as a DNA replication block both *in vitro* and within cells (6) (20, 21). A recent study by Potaman *et al.* has shown the propensity of the triplet repeat sequences (TRS) GAA-TTC repeats to form H-DNA and proposed pathways explaining replication blockage by such triplexes (22).

Generally tracts of guanine sequence can form quadruplex DNA (tetraplex) structure. In addition it has

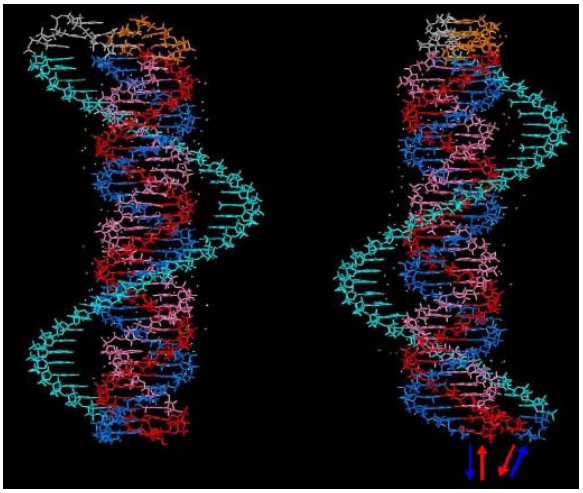

Figure 1. Example of a H-DNA (intramolecular triplex DNA) structure. One of the single strands (pink color) in the polypurine-pyrymidine tracts containing mirror repeat symmetry folds back and forms Hoogsteen hydrogen bonds to the purine strand of the duplex. The resulting structure in this half of the region is a triple stranded helix leaving the fourth strand as unpaired (shown in turquoish color).

been shown that CCG/CGG triplet repeats can also adopt tetraplex structures (2, 23). Hoogsteen hydrogen bonding is used for this guanine tetrad formation. The ability to form quadruplex DNA has been commonly described for single-stranded G-rich telomeric sequences *in vitro*. These structures also may act as a block for DNA replication or transcription. Quadruplex formation requires K+ or Na+, whereas Li+ and Cs+ prevent the guanine tetrad formation because they are too small or too large, respectively, to fit in the coordination site in the center of such quadraplexes (1, 9, 24).

Slipped DNA structures are formed from direct repeat sequences due to misalignment. Many direct repeat regions, such as telomeric DNA, or triplet repeat sequences, may undergo slippage. The single-stranded regions of such structures may interact to provide further stabilization (24).

RNA:DNA hybrids form during class switch recombination *in vitro* and *in vivo* and these have a classic R-loop conformation (25, 26). The RNA is generated by transcription, which is requisite for the R-loop formation. The displaced DNA strand, which is G-rich, appears to remain single-stranded DNA.

Recent examples of well-studied non-B DNA structures, which are pathologically relevant are described below.

4. NON-B DNA STRUCTURES IN DISEASE

4.1. Cruciform (hairpin) structures in diseases and genetic instability

Both in eukaryotes and prokaryotes, cruciform structures are considered as a source of genetic instability. Many independent human translocations have breakpoints at the center of AT-rich palindromic sequences on chromosme 11 and 22 (27, 28). The t(11;22) is the only known recurrent, non-Robertsonian translocation in humans. Some cases of this translocation lead to male infertility and recurrent abortion. Severely affected offspring develop Emanuel syndrome (5). In bacteria, when palindromic sequences containing AT-rich nucleotides are present on plasmid DNA, they can form cruciform structures. Such cruciform structures may lead to DSBs causing deletion, amplification, and recombination in bacteria (29, 30).

Deletions stimulated by a poly (R.Y) sequence from intron 21 of the polycystic kidney disease 1 gene (PKD1) or by long (CTG-CAG)n repeats in E.coli were suggested to occur due to cruciform structure (2, 8). The sequences at the junctions of the deletion indicated that the DSBs were generated close to or within sequences capable of adopting hairpin and/or slippage conformations.

Another example of non-B DNA structure in disease may be the extremely large block of chromosome-specific repetitive sequences (termed low copy repeats or LCR or duplicons). These blocks constitute a substrate for recurrent rearrangement associated with >40 human genomic diseases and are predicted to adopt non-B DNA conformations of substantial complexity and size (31, 32). It is shown that chromosome 22q11 is characterized by four LCRs. Some of the translocations involving this region of chromosome 22 have been shown to form cruciforms (31).

Triplet repeat tracts have been shown to form non-B DNA structures. Many hereditary neurological diseases are caused by the expansion of triplet repeat sequences in either coding or noncoding regions (33). Such triplet expansion is mediated by DNA replication, recombination or repair, and the precise mechanisms are still under investigation. DNA slipped structures and preferential formation of DNA hairpin structures, in addition to triplexes or tetraplexes appear to be important reasons for the genetic instability in these cases (33).

4.2. The bcl-2 Mbr forms a non-B DNA structure

The reciprocal translocation between chromosomes 14 and 18, referred to as t(14;18), occurs in follicular lymphomas and is the most common chromosomal abnormality in human cancer. The break at the immunoglobulin heavy chain locus on chromosome 14 is an interruption of the normal V(D)J recombination process. But the breakage on chromosome 18, at the bcl-2 gene, occurs within a confined region of 150 bp (called the major breakpoint region or Mbr) (Figure 2) (34-40). Recently, we have reported that the bcl-2 Mbr can form a non-B DNA structure within mammalian cells (41). We analyzed the structure formation within the cells or after extracting the chromosomal DNA out of the cells using nondenaturing conditions (41). In our studies, based on distinct patterns of bisulfite reactivity, we noted that independent non-B DNA structures may exist at peaks I and III within the Mbr (19, 41).

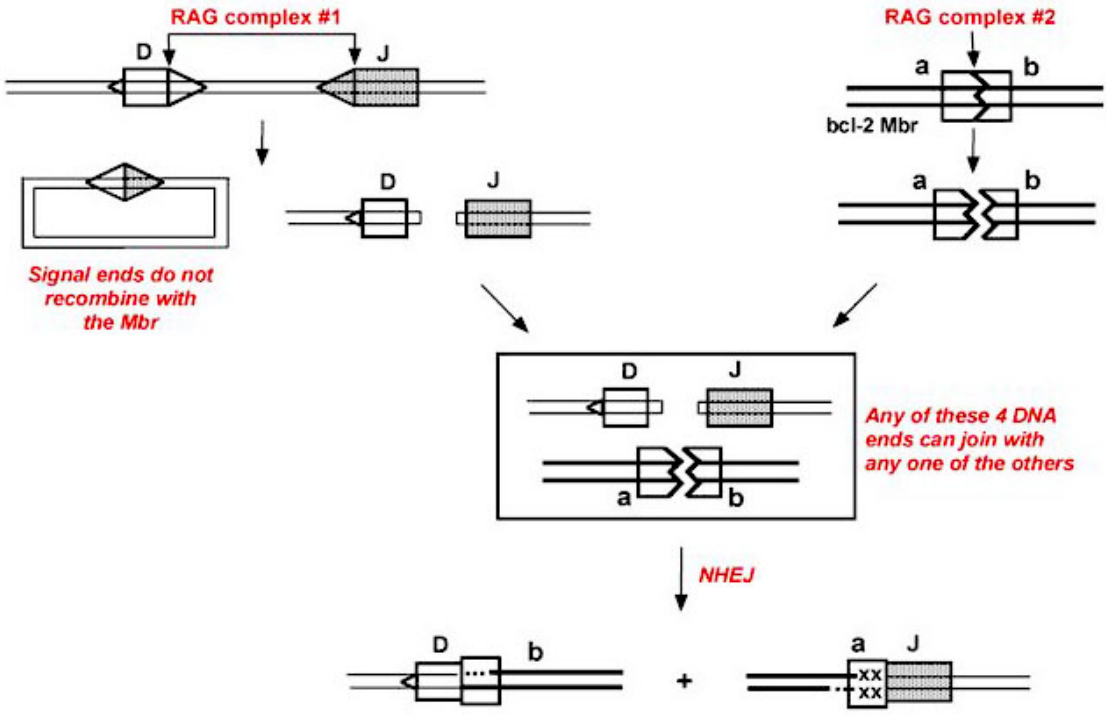

Figure 2. Biochemical model for the mechanism of the t(14;18) chromosomal translocation. Our studies indicated that the RAG cleavage at the Mbr (designated by the 'RAG complex #2' and the downward arrow) is independent of the RAG cleavage at the paired 12/23-signal pair (designated by 'RAG complex #1'). Coding ends join with bcl-2 Mbr using Ligase IV, indicating that the NHEJ pathway rejoins the broken DNA ends. The new findings from our most recent studies are shown in red color with italics.

We were also able to detect similar structure formation at the bcl-2 Mbr on cellular minichromosomes or on plasmid DNA when it is extracted using nondenaturing methods from mammalian cells or *E.coli*, respectively (41). Further studies using full molecule bisulfite sequencing (where both strands of the same molecule are analyzed), circular dichroism, and triplex specific antibodies in conjunction with electron microscopy indicate that the non-B DNA structure involved at this locus could be H-DNA (19). Though bcl-2 Mbr sequences at peaks I and III are purine/pyrimidine rich, there are mismatches as well. There are studies suggesting that certain mismatches are allowed or can be accommodated during triplex DNA formation (10, 42, 43). However, other possible non-B DNA structures resulting from, for example, replication slippage cannot be completely ruled out (44).

4.3. H-DNA in disease

Are there any other instances where triplex DNA results in a disease? Recently it was reported that DNA molecules containing two long GAA repeats can adopt a "sticky DNA" conformation, which is an intramolecular DNA structure adopted by triplex and is thought to be the cause for Friedreich's ataxia (45).

The authors provide evidence for two R.R.Y triplexes interacting together to form the sticky DNA on a plasmid. In different studies, an Y.R.Y triplex was proposed to form at similar Friedreich's ataxia sequences (22, 46). These studies show that GAA repeats can form triplex DNA and, hence, provide a potential explanation for Friedreich's ataxia.

Recently, using endogenous H-DNA forming sequences from the human c-myc promoter, it was shown that H-DNA is intrinsically mutagenic in mammalian cells. They also found DSBs near this H-DNA locus (47). Most of the Burkitts lymphoma patients carry a t(8;14) translocation, bringing together the c-myc gene of chromosome 8 and class switch enhancer region of IgH of chromosome 14. This chromosomal rearrangement leads to the overexpression of c-myc and lymphoma. It is well-known that during the t(8;14) translocation, many breakpoints in the translocated c-myc gene occur at the c-myc promoter region and downstream in exon 1 and intron 1 (48). It is possible that these findings are causally-related. In two independent studies, it was also shown that that PKD1 associated rearrangements could be mediated by triplex DNA (49, 50).

5. NUCLEASES THAT CLEAVE NON-B DNA STRUCTURES

Some evidence suggests that non-B DNA structures may cause a pause of the DNA polymerase, resulting in DNA nicks (6, 51). However, in many cases, the presence of a non-B DNA structure may not lead to genetic instability. Recently, we have seen that non-B DNA structures formed

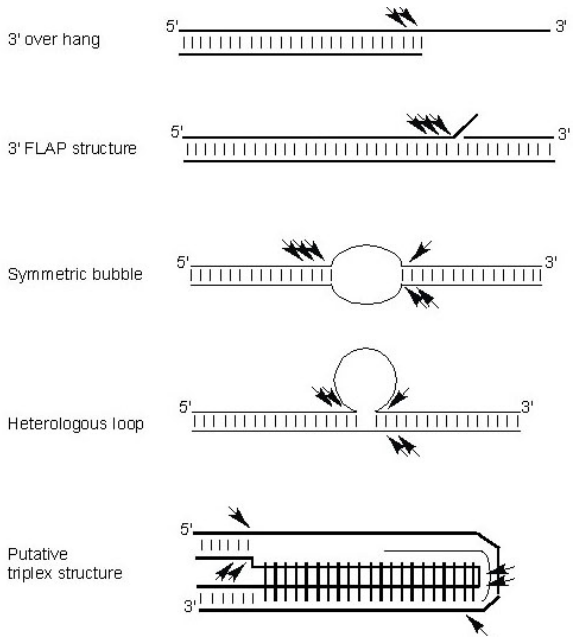

Figure 3. RAG cleavage activity at 3' overhang and non-B DNA structures under physiologic buffer conditions. RAGs cleave 3' overhangs in multiple locations at the duplex/single-stranded transitions (67). Our studies show that RAGs are able to cleave different non-B DNA structures such as symmetric bubbles, heterologous loop structures and a proposed triplex DNA structure at bcl-2 Mbr (53). Physiological concentrations of Mg2+ are used in all of the RAG cleavage studies summarized here.

at the bcl-2 Mbr can be cleaved by RAGs complex (a complex consisting of RAG1, RAG2, and HMGB1; also called V(D)J recombinase) leading to t(14;18) translocations and follicular lymphoma (Figure 2) (41, 52, 53). We also reported that in addition to this, RAGs cleave other non-B DNA structures such as symmetric bubbles or heterologous loops (Figure 3) (51, 53). RAG cleavage is mostly restricted to single- to double-stranded DNA transitions (Figure 3). In fact, we found that the RAG complex can cleave a bubble as small as 3 nt (51). In addition to the RAG complex acting as a structure- specific nuclease, RAGs can also catalyze transposition (also called RAG-mediated transposition) (54, 55). Recently, it was shown that cruciform structures on a plasmid DNA are a favored site for RAG-mediated transposition (56, 57). Though this could conceivably lead to genetic instability, instances of transpositional activation of an oncogene must be very rare because we are not aware of any examples of such events in human or murine studies.

Small deletions found at inverted repeats, probably due to hairpin structures are studied. It was found that Mre11/Rad50/Nns1 (MRN) complex in eukaryotes (*in vitro*) and SbcCD complex in bacteria recognize, cleave and process such hairpin structures (58-60). However, it has also been demonstrated that in *S.cerevisiae*, the complex is not responsible for the initiation of the break at the location of inverted repeats (61).

FEN-1, a structure-specific endonuclease is also thought to be involved in CNG triplet repeat stability (62). Deletions in other genes responsible for replication can also increase CNG repeat expansion or deletion. Examples include PCNA, RP-A, and the Bloom protein (BLM), a 3-5' helicase which reportedly interacts with FEN-1 in cleaving flaps (63-65).

There are also studies proposing that NER proteins in *E.coli* such as UvrB and UvrC are necessary for H-DNA-induced growth retardation and cell lysis. This conclusion was based on a study in which a 2.5 kb fragment of the PKD1 gene containing a polypurine-polypyrimidine tract was cloned into a plasmid and was then used for transformation into *E.coli*, which affected the host *E.coli* (4, 66). However, it remains to be seen whether NER proteins play roles in non-B DNA structure-induced genetic diseases in mammalian cells.

6. CNCLUDING REMARKS

The past 45 years of research have witnessed characterization of the structural conformations and properties of altered DNA structures. Recent findings that deviation from standard double-stranded DNA can lead to human disease have changed our views on the importance of non-B DNA structures. Many non-B DNA structures are now identified as causative agents for different cancers and other neurological disorders. However, in many of the cases, non-B DNA structure alone may not be the reason for a chromosomal breakage or alteration. Nucleases such as RAGs, MRN complexes, or others, which recognize various altered or branched DNA structures, may be important for the genomic alteration. Our recent finding that RAGs cleave the bcl-2 Mbr and other non-B DNA structures is important in this regard. The human genome sequences and genomic sequences from other organisms will help future research to focus on characterization and understanding of non-B DNA structures in other human disorders.

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