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Unusual DNA Structures.

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ABSTRACT

Duplex right handed DNA structure was established by Watson – Crick in 1953. Till 1979 researchers believed that DNA structure can only be right handed. But discovery of unusual left handed DNA structure in 1979 surprised the researchers. The Z- DNA structure was found to be zigzag and formed in the presence of high salt concentration. The presence of left handed DNA structure in genomic DNA give rise the presence of B-Z junction structure . The crystal structure studies of B-Z junction structure revealed that at junction one base pair bases are extruded. DNA also forms cruciform structures. The cruciform structures are formed in genomic DNA containing inverted repeats sequences. Genomic DNA containing long repeats of homopurine and homopyrimidine forms H- DNA or triplex DNA structures. The third strand of DNA forms Hoogsteen bonding. The quatret structure of DNA was known since 1962. But their function was not clear. The quatret structures are stabilized by monovalent or divalent cation and form right handed quadruplex structures . The unusual DNA structures have been found in vitro as well as in vivo. These unusual DNA structures are point of diseases where the reactions of harmful chemicals and enzymes take place. In this review article , the details of Z- DNA structure , B-Z junction structure , cruciform DNA structure , triplex structure and quadruplex DNA structure is described.

Keywords : Unusual DNA structures, Z-DNA structure , B-Z junction structure, cruciform structures, triplex structure, quadruplex DNA structure.

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INTRODUCTION

Structure of the right-handed B-form DNA has been known since 1953 [Watson et.al., 1953]. Instead of being a conformationally homogenous molecule, DNA has the capability of adopting several types of conformations as dictated by its sequence [Bacolla et.al., 2004]. It was later shown by atomic resolution single crystal X-ray diffraction analysis that the DNA hexamer d(CpGpCpGpCpG) forms a left-handed conformation (ZDNA) with altered helical parameters relative to the right handed B-form [Wang et.al., 1979]. This was followed by the identification of cruciform structures formed by inverted repeats [Lilley et.al., 1980 ; , Panayotatos et.al.,1981]. Cruciform structures, also known as Holliday junctions , occur when the four helices of two DNA duplexes are interconnected by strand exchange at a branch point, forming a 4-way, stacked X-shaped structure [Duckett , et.al. 1988; Liu, et.al., 2004; Watson, et.al., 2004; and Mirkin et.al., 2008 6] . The Z-DNA , cruciform of DNA , bent DNA have been found to exit in the living cells [Jaworski et.al., 1987; Payayotatos, et.al., 1987; Effron et.al., 1984; Marini, et.al., 1982 and Marini, et.al., 1984]. A family of unusual DNA structure has been discovered in segments with predominantly purines in one strand (pur.pyr sequences) . These sequences adopt an intermolecular triplex by minor repeats of G's and A's . It is likely that these unorthodox structures play an important role in the function of the eukaryotic genome. [Well, et.al., 1988]. The formation of higher order tertiary structures such as junctions, cruciforms and quadruplexes strongly depend on the DNA sequence. Finally, guanine-rich motifs in DNA were discovered to form parallel four-stranded complexes called tetraplex, Gquadruplex, or G4 DNA [Sen et.al., 1988]. iThe single-stranded G-rich DNA sequences have long been found to adopt a stable G-tetrad quadruplex structure in vitro [Henderson et.al., 1987; Patel et.al., 1999 and Parkinson et.al., 2002] . Its presence in vivo has also been established recently by the discovery of enzymes that can specially recognize such sequences to promote [Muniyappa et.al., 2002], cleave [Sun et.al., 2001 21], or unwind [Sun et. al., 1998 , Sun et.al., 1999 and Lin et.al., 2001] the G4-DNA structure.

More than ten different DNA conformations have now been discovered [Svozil et.al., 2008], and these are often referred to as secondary structures, alternative DNA, or non-B DNA. A non-B database has been developed for prediction of alternative DNA structures including Z-DNA motifs, quadruplex-forming motifs, inverted repeats, mirror repeats and direct repeats, and their associated subsets of cruciforms, triplex, and slipped structures, respectively [Cer et.al., 2011]. Non-B DNA structures are functional genomics elements that play a variety of roles in the cell [Zhao et.al., 2010]. These include gene function and regulation [Belotserkrvskii et. al., 2010], immune response [Collier et.al.,1988], telomere maintenance [Neidle et.al., 2003], recombination [Chin et.al., 2007], antigenic variation in human pathogens [Meier et.al., 1985], and the generation of genomic diversity [Smith et.al., 2010]. The DNA secondary structures are suggested to be involved in regulation at both transcriptional and translational levels; however, when the subtle balance between the replication, transcriptional, and repair machinery is impaired, these secondary structures may induce genetic instability. Alternate structure-forming sequences are known to be unstable and represent hotspots for deletion or recombination in bacteria, yeast, and mammals [Cromic et.al., 2000; Collick et.al., 1996; Grodeinin et.al., 1993; Branzei et.al., 2010]. This genetic instability has generally been related to DNA replication because non-B structures cause DNA polymerase pausing *in vitro* and replication fork pausing *in vivo* [Mirkin et al., 2007]. Slow replication was observed in an inverted repeat sequence in E*scherichia coli* [Leach et. al. 1994], and inverted repeats lead to deletions or chromosomal rearrangements more frequently in yeast that are deficient in DNA polymerase activity [Ruskin et.al., 1993 ; Lemoine et.al., 2005]. Slow progression of the replication fork could facilitate formation of secondary structures at long tracts of single-stranded DNA in the lagging-strand template [Sinden et.al., 1999]. These secondary structures pose obstacle to replication fork progression causing fork arrest and/or collapse ultimately leading to double-strand breaks (DSBs) and genome rearrangements [Wang et.al., 2006 ; Bacolla et. al., 2006]. The formation of alternative DNA structures can also activate nucleotide excision and SOS pathways resulting in segments of single-stranded DNA (ssDNA) [Bacolla et.al. 2011]. Such ssDNA regions can be converted to DSBs during replication and lead to mutations through mechanisms such as homologous recombination or nonhomologous end joining [Petermann et.al., 2010]. Conditions that favor the structural transitions from B-DNA to non-B DNA lead to genetic instability in model systems [Bacolla et.al., 2006]. Alternative structure-mediated mutagenesis has been implicated in the incidence of gross rearrangements and deletions as well as point mutations [Bacola et.al., 2007 ; Wang et.al., 1996; Wang et.al., 2004]. There is significant circumstantial evidence for the involvement of DNA secondary structures in association with genetic instability leading to human disease [Wells et al. 2009; Bacolla et.al. 2009 ; Sharma et.al., 2011].

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Left – Handed DNA (Z- DNA)

In 1979 the crystal structure of a DNA molecule was published [Wang , et.al.; 1979]. Although it was already known that nucleic acids could adopt various alternative conformations, this crystal structure was concrete evidence of the left-handed helical form of DNA. This DNA conformation was called Z-DNA because of its zigzag, sugar-phosphate backbone. Interest in Z-DNA has grown in time as additional evidence for its in vivo existence has accumulated. Unlike right-handed nucleic acids, Z-DNA is highly immunogenic, and both monoclonal and polyclonal antibodies were raised against Z-DNA (Lafer , et.al.; 1981 , Mollar , et.al., 1982). Antibodies allowed different groups to show that Z-DNA does exist in vivo and also to map several regions more prone to be in such conformation [Arndt-Jovin, et.al., 1983, Nordheim , et.al., 1981, Lancillottti, etal., 1987, Lipps et.al. 1983]. Recently, bioinformatics tools have been developed [Li et.al., 1983] that predict several genomic hot spots for Z-DNA formation and suggested roles for such structures. Significant experimental evidence regarding the in vivo presence of Z-DNA has accrued, but its biological role has not been fully elucidated. Considerable progress has been made in discovering proteins that bind Z-DNA with great specificity. At present, four proteins able to bind the Z form of nucleic acids have been described: the IFN-induced form of the RNA editing enzyme ADAR1, the innate immune system receptor DAI (also known as DLM-1 and ZBP1), the fish kinase PKZ, and the poxvirus inhibitor of IFN response E3L [Herbent , et.al.; 1995, Schwartz , et.al.; 2001, Rothenburg , et.al.; 2005, Kim,et.al.,2003]. These proteins all have important roles in physiological and pathological processes related to the IFN system. In addition, they share Z-DNA binding ability because of a common, winged helix-turn-helix (WHTH) domain named Zα. Isolated Zα domains from all of the above-mentioned proteins have been crystallized in complexes with short stretches of DNA duplex $(CG)_3$ and shown to bind DNA in the left-handed Z form in a very similar fashion [Schwartz , et.al.; 2001, Schwartz , et.al.; 1999, Kim , et.al.;2009, Ha, et.al.; 2004, Ha et.al.; 2008]. The Zα domain of ADAR1 was the first to be discovered and is the best characterized of all Z-DNA binding proteins. ADAR1 is an adenosine deaminase responsible for the A-to-I editing of RNA and exists in two alternative isoforms, one expressed constitutively and the other induced by IFNs. The IFN inducible form of ADAR1 possesses two WHTH domains, Zα and Zβ, the former of which became the prototype of all the Z domains and later was shown to bind Z-RNA as well [Placido et. al.; 2007]. Although Zβ shows the same topology as other Z domains, it does not bind left-handed nucleic acids [Athanasiadis, et.al.; 2005]. Apart from interest in the protein itself, Zα-ADAR1 is also a powerful tool to study left-handed nucleic acids structures, because it is able to strongly stabilize such structures under nearly physiological conditions. Most of the structural and in vitro biochemical studies on Z-DNA have been performed with short stretches of DNA and mostly with plain CG repeats. CG repeats are used because this sequence undergoes the B-to-Z structural transition most readily. Unlike the right-handed B form, the repeating unit of Z-DNA is a dinucleotide and in particular a purine-pyrimidine repeat. Whereas such short oligonucleotides have been good starting models because of the ease with which they can support the B-to-Z transition, they do not accurately reflect the context in which Z-DNA may form in vivo. Long stretches of perfect CG repeats are infrequent in genomes because they represent hot spots of genomic instability [Wang , et.al.; 2006a]. Instead, in genomic sequences Z-DNA forming sequences are frequent in which the pattern of the Pu-Py dinucleotide repeat is broken by Pu-Pu or Py-Py dinucleotides.

The term Z-DNA stems from the observed zig-zag conformation of the phosphate backbone of a left-handed helix taken up by alternating purine-pyrimidine DNA sequences (GC repeats) under high salt conditions [Wang, et .at.; 1979]. Here, due to the displacement of the base pairs away from the axis, only one groove can be observed that is analogous to the minor groove of B-DNA. The bases forming the major groove in B-DNA are reorganized in Z-DNA as such that they build a convex outer surface (Figure 1). Each guanine base is rotated around the glycosidic bond into *syn*-conformation, with the sugar puckered in C3' endo-conformation, while the cytosine in the adjacent base step is in the C2' endo, *anti*-conformation. Compared to A- and B-DNA, Z-DNA possesses a helical diameter of 18 Å, with 12 bp per helical turn, a rise of 3.7 Å and rotation by 30° per bp. In addition, the phosphate groups are closer together. Due to the electrostatic repulsion of the phosphate groups and the energy penalty associated with rotation of the Gs into *syn*-conformation, under physiological conditions the Z-conformation is the less favored higher energy state and the DNA is pushed into the B-form. This also explains why Z-DNA becomes the stable conformation under high-salt concentrations, since the salt decreases the electrostatic repulsion of the phosphate [Dickerson, et.al.; 1992, Rich et.al.; 2003].

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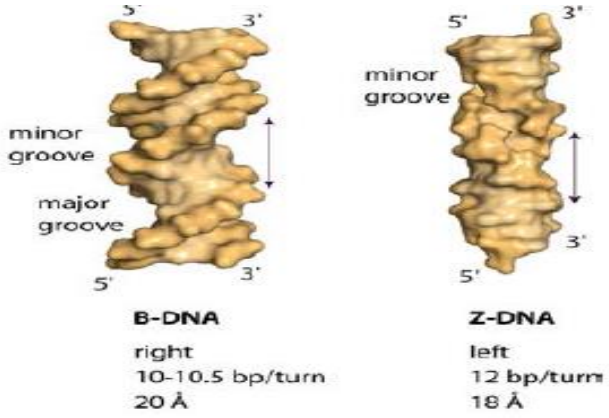

Figure -1 Structure of B and Z DNA

B-Z Junction

When a region is converted to Z-DNA within a long continuous double stranded B-DNA, two B-Z junctions will be formed flanking the region of Z-DNA. Many studies have been carried out to characterize the B-Z junction using biochemical and biophysical methods, however, the structural properties of B-Z junction have not been well studied. It is difficult to prepare a continuous hybrid B/Z DNA duplex containing both B and Z conformations in physiological conditions, which is necessary for structural analysis at high resolution. A 15-base pair dsDNA consisting of two regions: one is CG rich and can be easily converted to Z-DNA in Z-DNA inducing conditions, and the other region is TA rich and maintains B conformation predominantly (Figure-2) was designed . Therefore, in the presence of the hZαADAR1, one B-Z junction is thus formed in the middle of the DNA duplex, connecting the Z- and B-DNA. The hZαADAR1 domain (aa.140-202) was co-crystallized with the DNA [5'-GTCGCGCGCCATAAACC-3' and 5'- ACGGTTTATGGCGCGCG-3'] [Ha, et.al.; 2005]. Overall structure of the dsDNA is composed of eight base-pair Z-DNA, six base-pair B-DNA and the B-Z junction (Figure 3&4). The Z-DNA is stabilized by four Z-DNA binding domains (hZαADAR1, Figure -3) and shows standard base-pair step parameters of Z-DNA (Figure-3&4). B-DNA also has standard basepair step parameters of B-DNA (Figure-3&4). At the B-Z junction, A and T bases are extruded from the duplex, thereby linking left-handed Z-DNA to right-handed B-DNA. The base-pair step parameters of BZ junction have 3.82 Å rise (Dz) and -16.9º twist (Figure-3&4). Though one junctional base is extruded, base stacking is continuous from Z-DNA to B-DNA through the B-Z junction without significant impairment. Thus, base stacking is also a major stabilizing factor even at the B-Z junction as well as in B-DNA and Z-DNA regions (Figure. 3), [Ha,et.al.; 2005, Drew et.al.; 1979, Wang, et.al.; 1979]. It seems that extrusion of a base from each strand accommodates a reversal in the helical direction of the

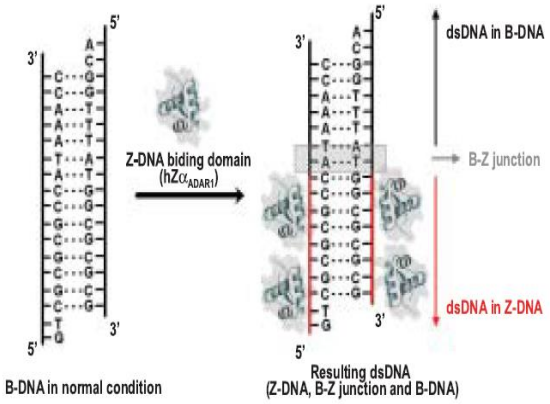

Figure- 2 The strategy used of making a stable junction between B- DNA and Z- DNA . Four DNA binding proteins from human ADAR1 (hZαADAR1) stabilize the Z- conformation in one –half of a 15 base pair ds DNA and other region remains as a junction and B- DNA

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Figure-3 Skeletal stereo views of DNA .and B-Z junction sequence.backbone from B- to Z conformation. It is also speculated that these extruded bases may be specific recognition sites for enzymes mediating DNA modification [Ha,et.al.; 2005].

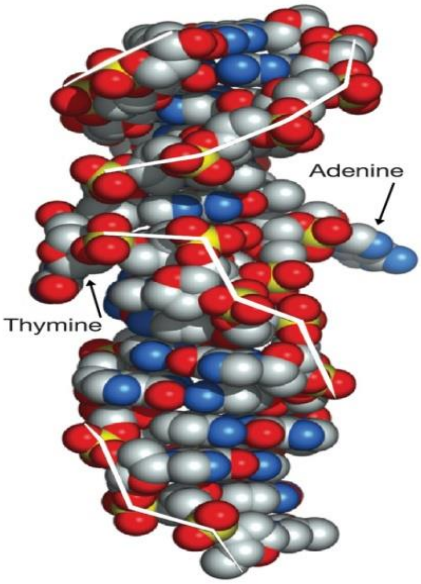

Figure 4 Space filing model of B-Z junction

Hairpins and Cruciforms

Hairpin structures can be formed by sequences with inverted repeats (IRs), also termed palindromes. There are two mechanism , in the C-type pathway, a substantial region of dsDNA is denatured, allowing the folding of the whole hairpins on both strands in one step. In the S-type pathway, a small region is denatured (~10 bp), allowing the folding of a small hairpin that can then be elongated through branch migration (Figure-5).

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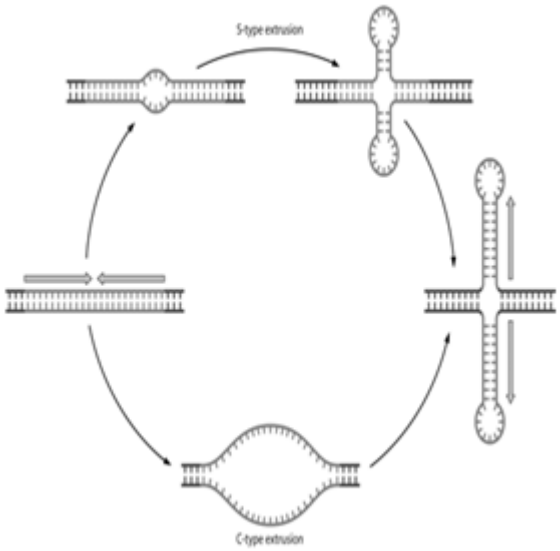

Figure -5 Mechanisms of cruciform extrusion [Bikard, et.al.; 2010]

A cruciform structure is formed by intrastrand base pairing of inverted repeat sequences and is characterized by the presence of a four-way junction in which two of the branches are hairpin structures formed on each strand of the inverted repeat [Lilley et.al., 1980] (Figure 5). The bases located between the inverted repeats do not self-pair and instead form the apical loops of the hairpins; however the overall structure is stabilized by the free energy of negative super coiling [Lilley et.al., 1980]. Cruciform is structurally similar to a Holliday Junction (HJ) recombination intermediate [Oussatcheva et. al., 2004; Smith et.al., 2008]. The existence of cruciforms has been demonstrated *in vitro* [Karrer et.al.,1976] and *in vivo* [Sinden et.al., 1985; Panayototos et.al., 1987a; Zannis-Hadjopoulos et.al., 1988]. Cruciform structures have been reported in the genome of *E. coli* [Mcclellan et.al.,1990] as well as mammalian cells [Zannis-Hadjopoulos et.al., 1988]. Cruciform-forming inverted repeat sequences have been found at the operator and transcription termination regions [Rosenberg et.al., 1979], as well as at the replication origin region [Zannis-Hadjopoulos et.al., 1988 ; Lockshon et.al., 1988]..

The distribution of such sequences often overlaps with chromosomal regions prone to gross rearrangements [Lockshon et.al., 2007]. Because cruciform structures are energetically unfavorable, they are thought to form transiently *in vivo* as stable structures. The action of cellular factors such as junction specific nucleases, binding proteins, and DNA helicases is suggested to affect the equilibrium and the rate of formation of cruciform structures *in vivo* [Mizuuchi et.al., 1982].

Triple Helices and H-DNA

Observations by Larsen & Weintraub [Larsen , et.al.; 1982] and Hentchel [Hentchel, et.al.; 1982] that promoters of eukaryotic genes in both active chromatin and super coiled plasmids show hypersensitivity toward S 1 nuclease indicated to many researchers that unusual structures might be formed at those sites [Weintraub , et.al.;1983]. Numerous studies revealed that S1 sensitivity was associated with homopurine-homopyrimidine stretches [Wells , et.al.;1988]. This finding was surprising because homopurine-homopyrimidine sequences could adopt neither cruciform nor Z-DNA structures. Slippage loops [Hentchel, et.al.; 1982, Mace et.al.; 1983, Mekeon, et.al.; 1984, Shen , et.al.; 1983], left-handed helices [Cantor, et.al.; 1984, Margot, et.al.; 1985, Pulleyblank , et.al.; 1985 106,107,108), triple helices (Christophe , et.al.; 1985, Lee, et.al.; 1979], and quarter helices [Johnson , et.al.; 1978] had been discussed in literature; but the controversial data on fine mapping of S1 cleavage sites did not permit definite conclusions about the nature of any unusual structures in the homopurine-homopyrimidine tracts. Such conclusions were finally enabled by two-dimensional gel electrophoresis of super coiled DNAs carrying a homopurine-homopyrimidine insert [Lyamichevi, et.al.; 1985 , Lyamichevi, et.al.; 1986, Mirkin, et.al.; 1987]. These studies revealed beyond any doubt that an unusual structure was formed and, remarkably, that this structure was stabilized by hydrogen

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ions--hence the name "H form" for the unusual structure (Figure 6). The main element of the H form an intramolecular triple helix formed by the entire pyrimidine strand and half of the purine strand; the other half of the purine strand remains single stranded. The triple helix is stabilized by CG*C+ and TA*T base triad (Figure 7).

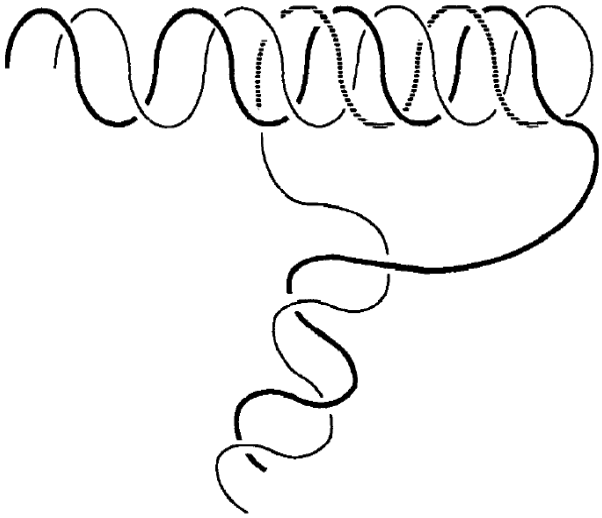

Figure- 6 H-DNA model. Bold line, homopurines strand; thin line, homopyrimidine strand; dashed line, the half of the homopyrimidine donated to the triplex.

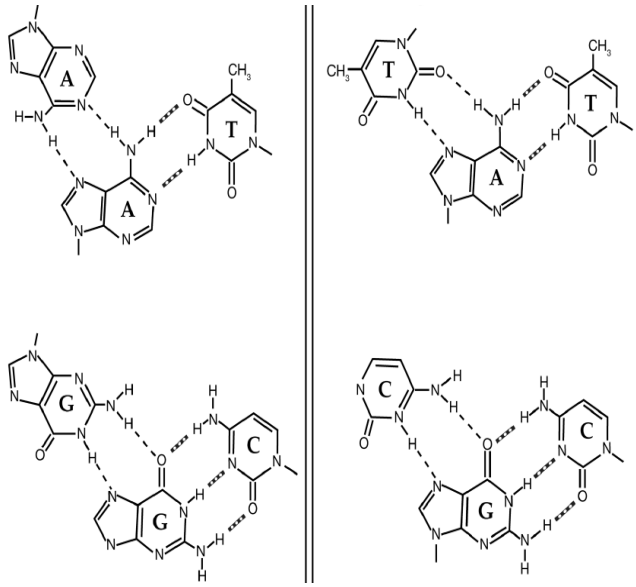

Figure-7 Schematic representation of canonical base triplets formed in purine (TA*A, CG*G ,left) and pyrimidine (TA*T , CG^*C^+ , right) triplex motif. Watson – Crick base pairing is illustrated by dotted lines , and Hoogsteen base pairing by broken lines.

H-DNA was the first example of an intramolecular DNA triplex, It has become clear in recent years that an entire family of H-like DNA structures exists whose members differ in the chemical nature of their triplexes; such structures can exist in different isomeric forms depending on ambient conditions and sequences. Similarly, the variety of sequences known to be able to adopt the H-DNA structure has significantly increased during the last few years. The N3 position of cytosine seemed the most probable protonation site. Among free nucleotides it has the highest pK value for protonation, and this pK value had been known to increase substantially when protonated cytosines were involved in different structures.. A model of H form DNA was proposed by Lyamichev et al (Lyamichevi, et.al.; 1986) (Figures 8a and 8b). It consists of an

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intramolecular triple helix formed by the pyrimidine strand and half of the purine strand; the other half of the purine strand is single stranded. As Figure 8a shows, this structure is topologically equivalent to unwound DNA.

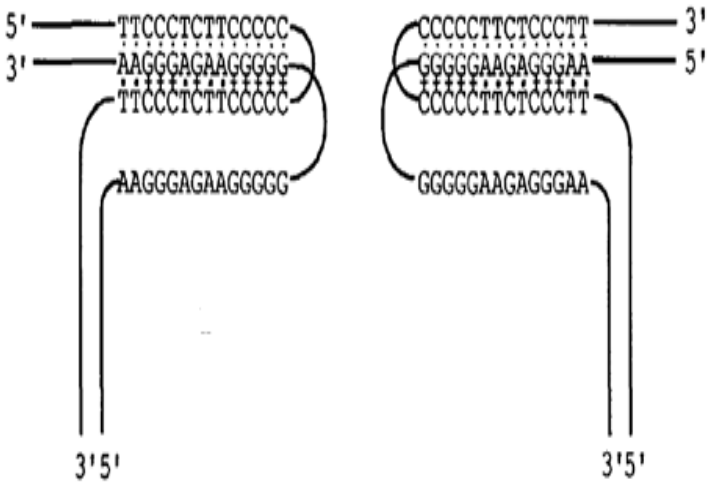

Figure 8 Two isoforms of H-DNA . Watson-Crick hydrogen bonds are labeled points, nonprotonated Hoogsteen hydrogen bonds are shown by squares, and protonated Hoogsteen hydrogen bonds are shown by plus symbols.

Two isoforms of H form are possible: one single-stranded in the 5' part of the purine strand and the other single-stranded in the 3' part . The existence single-stranded purine stretches in H-DNA may explain its hyper reactivity to S1 nuclease. TA*T and CG*÷C base triads stabilize the triple helix . Thymines or protonated cytosines from the third strand interact with adenines or guanines, respectively, from AT or GC base pairs via Hoogsteen rules (Hoogsteen, et.al.;1963). The protonation of cytosines is crucial for the formation of CGC÷ base triads. This observation explains the pH dependency of the structural transition. It is also clear that only one half of the cytosines must be protonated for Hoogsteen hydrogen bonding, while the remaining cytosines form Watson-Crick hydrogen bonds.

It is important that the triads be isomorphous such that good stacking in a triple helix is possible. The formation of triplexes, first suggested by Felsenfeld et al [Felsenfeld,et.al.,1957] for mixtures of homopurine and homopyrimidine polyribonucleotides, has been documented further in many studies [Lee, et.al., 1979 ; Morgan , et.al., 1968]. The H-DNA model suggested several obvious predictions. First, it should be true for several simple repeats including d(G)n-d(C)n d(A)n.d(T)n. For d(A)n.d(T)n, one would expect a pH-independent structural transition, since TAT triads do not require base protonation. However, for a long time all attempts to detect this transition remained unsuccessful. Using single-strand-specific nucleases and chemicals as probes, Fox [Fox, et.al.; 1990] found that d(A)n.d(T)n stretches adopt the H conformation under the influence of DNA super coiling at pH 8. A less trivial prediction regarding sequence requirements of H-DNA formation is based on the importance of TA*T and CG*C÷ triad isomorphism. When the pyrimidine-rich strand folds back to form a triplex, cytosines from one half of the homopurine-homopyrimidin sequence should interact with GC but not AT base pairs in its other half. Conversely, thymines in one half should interact with AT but not GC base pairs from the other half. Thus, a homopurine-homopyrimidine sequence must be a mirror repeat to form H-DNA. Regular sequences d(G-A)n.d(T-C)n, d(G)n.d(C)n, and d(A)n.d(T)n are mirror. One would expect that irregular homopurine-homopyrimidine sequences with mirror symmetry must adopt H conformation as well. This hypothesis was proved by Mirkin et al [Mirkin , et.al.; 1987] in studies of cloned [Sergei, et.al.; 1994] DNA.

Triplexes have been shown to exist in chromosomes and nuclei, and the existence of H-DNA structures has been evidenced both *in vitro* and *in vivo* [Zain et al 2003]. Triplex formation *in vivo* is supported by the identification of mammalian proteins that bind specifically to them [Nelson et.al., 2000; Musso et.al., 2000] and to the polypyrimidine [Gracia Bassels et.al., 1999] and polypurine single strands [Sharma et.al., 2011]. H-DNA conformations have been identified *in vivo* by using triplex-specific monoclonal antibodies [Lee

et.al., 1987 ; Raghavan et.al., 2005] and fluorescent "in situ nondenaturing" hybridization [Ohno et.al., 2002]. The presence of an H-DNA conformation *in vitro* has been demonstrated in constructs containing the sequences of interest from *E. coli* and mammalian genomic DNA or by using chemicals that modify nucleotides specifically in single-stranded DNA or double-stranded DNA [Mirkin et.al., 1994 ; Raghavanet.al., 2006]. The sequence-specific DNA recognition and binding characteristics of synthetic TFOs have been extensively studied because of their potential applications in genome modification and therapy [Seidman et.al., 2003]. Most annotated genes in both the mouse and human genomes are predicted to contain at least one unique potential TFO binding site [Gaddis et.al., 2006]. Similarly, naturally occurring sequences capable of adopting H-DNA structures are very abundant in mammalian cells (1 in every 50,000 bp in humans) [Jain et.al., 2008; Schroth et.al., 1995]. Majority of polypurine·polypyrimidine sequences are located in introns, promoters and 5- or 3- untranslated regions and are enriched in genes involved in cell signaling and cell communication [Bacolla et.al., 2006a]. Importantly, H-DNA structure-forming sequences are found flanking protooncogenes [Kang et.al., 1992; Smith et.al., 1992; Todd et.al., 2005].

The triplexes described above are intra strand triplexes found in the natural genomic sequences. However, inter strand triplexex are also formed. Intermolecular triplexes are formed when the triplex-forming strand originates from a second DNA molecule. Intermolecular triplexes have attracted much attention because of their potential therapeutic application in inhibiting the expression of genes involved in cancer and other human diseases, for targeting disease genes for inactivation, for stimulating DNA repair and/or homologous recombination pathways, for inducing site-specific mutations, and for interfering with DNA replication. An example of triplex formation with a polypurine TFO sequence specific for the human *c-MYC* P2 promoter is shown in Figure 9

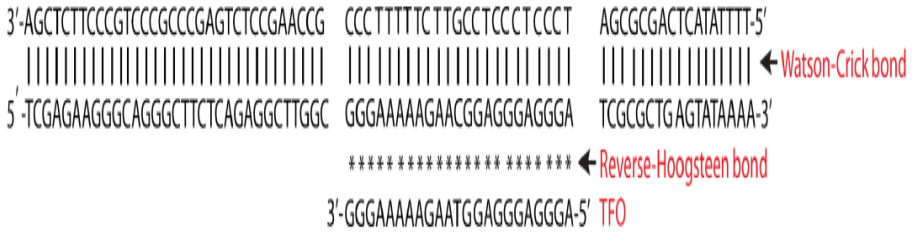

Figure-9 Triplex forming sequences in the human c-MYC gene. The TFO placed in an antiparallel orientation relative to the target duple from the human c-MYC P2 promoter . Vertical lines indicates Watson – Crick hydrogen bonds and stars indicates reverse Hoogsteen hydrogen bonding.

Triplex formation occurs in two motifs, distinguished by the orientation of the third strand with respect to the purine-rich strand of the target duplex. Typically, polypyrimidine third strands (Y) bind to the polypurine strand of the duplex DNA via Hoogsteen hydrogen bonding in a parallel fashion (*i.e.* in the same 5' to 3', orientation as the purine-rich strand of the duplex), whereas the polypurine third strand (R) binds in an antiparallel fashion to the purine strand of the duplex via reverse-Hoogsteen hydrogen bonds [Beal, et.al., 1991; Letai, et.al. 1988; Durland, et.al.,1991]. In the antiparallel, purine motif, the triplets are G:G-C, A:A-T, and T:A-T; whereas in the parallel, pyrimidine motif, the canonical triples are C+:G-C and T:A-T triplets (where C+ represents a protonated cytosine on the N3 position) The hydrogen bonding schemes found in purine and pyrimidine motif triplexes are depicted in Figure 7. Antiparallel GA and GT TFOs form stable triplexes at neutral pH, while parallel CT TFOs bind well only at acidic pH so that N3 on cytosine in the TFO is protonated [Lee ,et.al., 1979], substitution of C with 5-methyl-C permits binding of CT TFOs at physiological pH [Lin , et.al., 1994; Singleton , et.al., 1992] as 5-methyl-C has a higher pK than does cytosine. For both motifs, contiguous homopurine-homopyrimidine runs of at least 10 base pairs are required for TFO binding, since shorter triplexes are not substantially stable under physiological conditions, and interruptions in optimum sequence can greatly destabilize the triplex structure [Cheng, et.al., 1993; Orson, et.al., 1999; Cheng, et.al., 1992;Aich, et.al., 1998; Gowers, et.al., 1997]. If purine bases are randomly distributed between two duplex strands, the consecutive third-strand bases should switch from one strand of the duplex to the other, resulting in a structural distortion of the sugar-phosphate backbone and lack of stacking interactions. This is energetically unfavorable, and therefore the most appropriate duplex target for triplex formation contains consecutive purine bases in one strand. Thus, an ideal target for triplex formation is the presence of a homopurine

sequence in one strand of duplex and a homopyrimidine sequence in the complementary strand. Triplex formation is kinetically slow compared to duplex annealing [Vasquez , et.al., 1995; Alberti, et.al., 2002; James, et.al., 2003; Rusling, et.al., 2008; Paes, et.al., 1979] . However, once formed, triplexes are very stable, exhibiting half-lives on the order of days [Vasquez, et.al., 1995; James, et.al., 2003]. Formation of both intramolecular and intermolecular triplexes depends on several factors including length, base composition, divalent cations, and temperature [Frank-Kamenetskii , et.al., 1995 7]. The affinity and specificity of TFO binding are critical features to their success of a gene targeting molecules. The dissociation constants (Kds) of TFOs for their target duplexes typically range from 10−7 to 10−10 M, making them feasible gene targeting agents [Jain , et.al., 2008]

Quadruplex Structures

Quartet : A G-quadruplex compact structure is formed when four guanines from different places along a G-rich strand are held together by a special type of hydrogen bonding . The G-quadruplex structures have been prepared in the laboratory by the folding of synthetic DNA strands rich in guanine . The G-quadruplex structures assemble into higher order structures. The higher order structures are called guanine quadruplexes. The G-quadruplex structures result from the hydrophobic stacking of two or more G-quartets. The exhibit G-quadruplex possess right-handed helicity. In the quadruplex structure four guanines establish a cyclic array of hydrogen bonds from the Watson–Crick and the Hoogsteen faces (Fig. 10a) the hydrogen bonding scheme is shown in Figure 10b. A G-quartet is a planar association of four guanines held together by four central H-bonds between H1 of nitrogen and O6 of carbonyl group and also by four external H-bonds between H2 of amine and N7 (Fig.10c). A space filling model reveling the hollow center is shown in Figure 10d. The nucleoside sugars (ribose or deoxyribose) can adopt several conformations. The two most favorable sugar conformations are C2'-endo (found in most DNA G-quadruplexes) and C3'-endo (found in most RNA structures) (Fig. 10e) [Smith, et.al.; 1994]. The guanine bases are covalently linked to the sugar via the glycosidic bond that can display two major different torsion angles: syn and anti (Fig.10f). The anti conformation can be formed whatever the sugar conformation (C2'- or C3'-endo), whereas the syn position is unfavorable in the case of C3'-endo sugar conformation because of the steric hindrance between O3' and C5'. Despite this steric effect, the syn position is possible for guanine. Rotation around the glycosidic bond enables a guanine base to interconvert between syn and anti conformation is shown in Figure 10g. Each G-quadruplex presents four grooves defined by two neighboring guanines in a G-quartet. According to their respective syn or anti conformations, grooves can be wide, medium, or small [Smith , et.al. 1992], contributing to the conformational diversity of G-quadruplex structures. However, this diversity is more limited in the case of tetra molecular quadruplexes; when all strands are parallel, all guanine in a quartet must adopt the same glycosidic bond angle, leading to all-syn or all-anti quartets (the latter being the general case), and all grooves are of medium size. The interplay between glycosidic torsion angles and strand polarities , indicated by (+) or (-) , give rise to different widths is shown in Figure 4.10h. Based on theoretical calculations it was once suggested that tracts of guanines favor formation of G-quadruplexes. Quadruplexes with four parallel strands in which all guanines adopt the *anti* conformation and that alternating *anti*/*syn* arrangements were restricted to intramolecular G quadruplexes with antiparallel strands [Mohanty , et.al.; 1993]. It is easily realized that any G-quadruplex structure that contains antiparallel strands must have bases in both the *anti* and *syn* conformation in order to maintain the tetrad base-pairing scheme. There are examples of virtually any combination of glycosidic torsion angles. Several structures with exclusively *anti* [Aboul –ela, et.al.; 1992 , Wang , et. al.; 1992, Wang, et.al.; 1993] or *syn* [Henderson , et.al.; 1987] conformations have been characterized, as have structures with regularly alternating *anti*/*syn* conformations [Balagurumoorty et.al.; 1992, Kang, et.al.; 1992, Smith , et.al.; 1992, Smith , et.al.; 1992, Greene, et.al.; 1995, Catasti, et.al.; 1996) or mixtures of *anti*/*syn* conformations within both guanine tracts and guanine tetrads [Greene, et.al.; 1995, Catasti, et.al.; 1996. , Smith , et.al.; 1994 , Strahan , et.al.; 1994]. The stacking of G-quartets is stabilized by monovalent (Rb^+, $NH4^+$, K^+, Na^+, Cs+, Li^+), divalent (Sr^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}), or trivalent ($Tb^{3+,}$ $Eu^{3+)}$ [Wang , et.al.; 1995 , Galezowska, et.al.; 2007 , Worlinsky, et.al.; 2009] cations according to their ionic radii and their hydration energies. The order of cation ability to stabilize and/or to induce G-quadruplex structures is as follow: $K^+ > NH4^+ > Rb^+ > Na^+ > Cs^+ > Li^+$ for monovalent cations [Hupper et.al.; 2005, Wong , et.al.; 2003] and $Sr^{2+} > Ba^{2+} > Ca^{2+} > Mg^{2+}$ for divalent cations [Venczel , et.al.; 1993]. G-quartet may be stack upon each other to form G-quartet core in presence of metal ion (Figure- 4.1i) . The ion is bound between two G-quartets , co-ordinated (dashed lines) by eigth guanine O6 oxygen.

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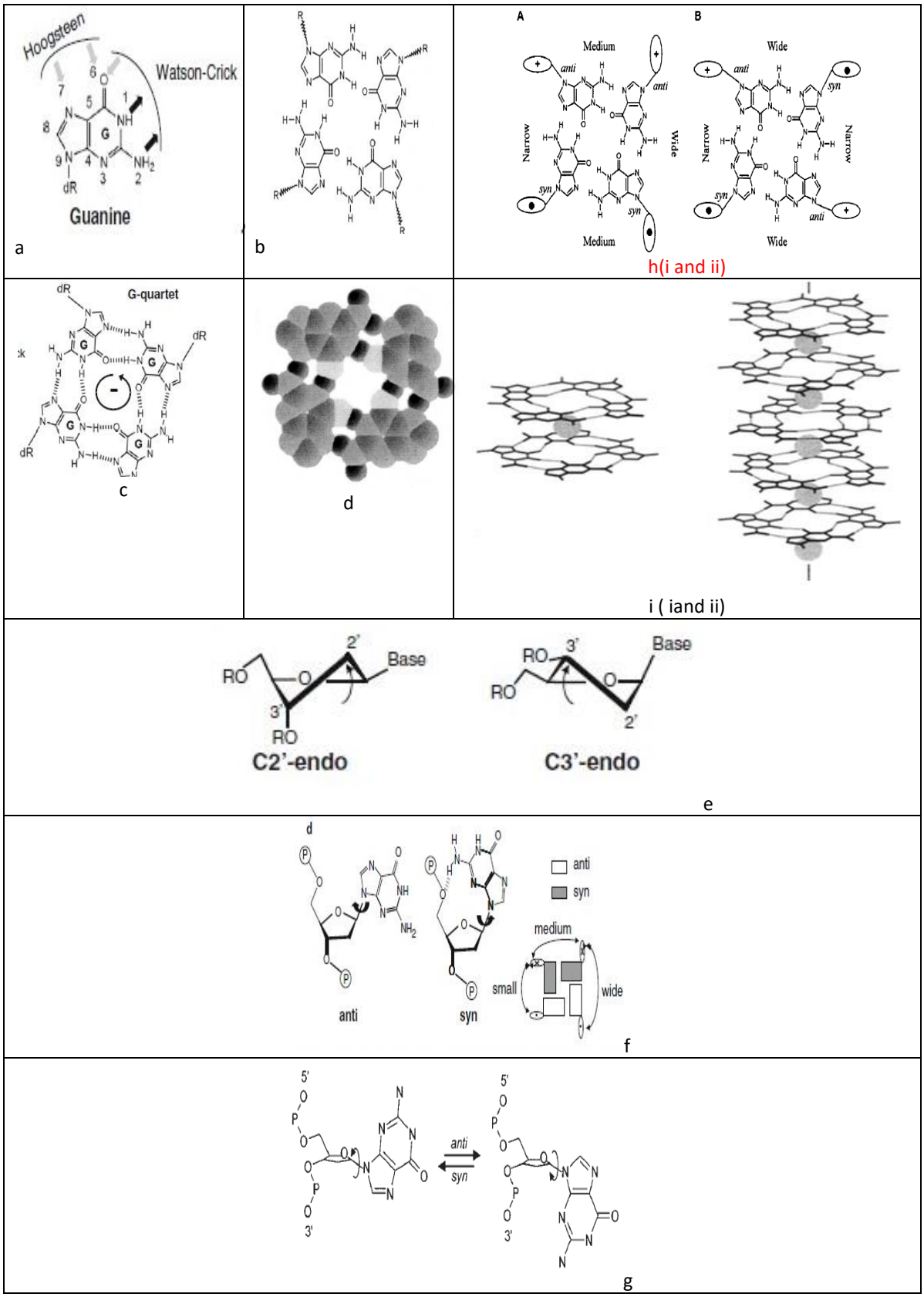

Figure-10 Guanine and G-quartet (a) chemical formula of canonical guanine and two hydrogen bonding faces (Watson – Crick and Hoogsteen) which are implicated in G-quartet formation. Arrows indicate H-bond donors (in black) and acceptors (in gray), (b) the hydrogen bonding scheme in G- quartet (c) Classical G-quartet structure with anticlockwise rotation (-) of the donor NH to the acceptor C=O hydrogen bonds, (d) (c) Two most favorable sugar configurations og

guanine of quarter : C2' or C3' endo, (d) A space filling model reveling the hollow center, (e) Two torsion single of guanine glycosidic bond (syn and anti) that determine groove dimension of G- quartet : wide , medium and small, (f) anti and syn conformation of guanine glycoside bond (g) Rotation around the glycosidic bond of a guanine base to interconvert between syn and anti conformation , (h) The interplay between glycosidic torsion angles and strand polarities , indicated by (+) or (-) , give rise to different widths. (h-i) guanine tetrads with two pairs of adjacent parallel strands that have identical glycosidic torsion angles generated two medium groove , one wide groove , and one narrow groove , (h-ii) guanine tetrads with exclusively alternating antiparallel strands have two wide and two narrow grooves, (i) Stacking of G- quartet core in presence of metal ion.

The human genome contains nearly 376 000 distinct sites with the potential to form G4 DNA [Todd et al., 2005; Huppert et.al., 2005; Sharma et. al., 2011], and the evidence for *in vivo* formation of G4 DNA has emerged in recent years [Lipps et. al., 2009]. Notably, G4 DNA has been observed by electron microscopy from transcribed human G-rich DNA arrays in bacteria [Duquette et.al., 2004] and by immunochemistry at the end of the ciliate Oxytricha telomeres [Paeschke et.al., 2004]. The G-rich chromosomal domains predicted to form G4 DNA include four classes of repetitive regions: telomeres, rDNA, immunoglobulin heavy chain switch region, and G-rich minisatellites [Hershman et.al., 2008]. Replication, recombination, transcription, and telomeric DNA elongation involve steps in which two strands of duplex DNA can be unwound transiently, providing an opportunity for the G-rich strand to form quadruplex structures during these DNA metabolic events [Lipps et.al. 2009]. Formation of G4 DNA modulates key cellular processes such as immunoglobulin gene rearrangement, promoter activation, and telomere maintenance [Meizels et. al., 2006].

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