REVIEW

Z-DNA in the genome: from structure to disease

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Abstract

The scope of studies investigating the architecture of genomic DNA has progressed steadily since the elucidation of the structure of B-DNA. In recent years, several non-canonical DNA structures including Z-DNA, G-quadruplexes, H-DNA, cruciform DNA, and i-motifs have been reported to form in genomic DNA and are closely related to the evolution and development of disease. The ability of these structures to form in genomic DNA indicates that they might have important cellular roles and are therefore retained during evolution. Understanding the impact of the formation of these secondary structures on cellular processes can enable identification of new targets for therapeutics. In this review, we report the state of understanding of Z-DNA structure and formation and their implication in disease. Finally, we state our perspective on the potential of Z-DNA as a therapeutic target.

Keywords Z-DNA · Disease · Z-DNA-binding protein · Therapeutic target · Transcription

Introduction

The main backbone of the human genome is comprised of double-stranded DNA (dsDNA) in the canonical B-DNA conformation with a right-handed helix (Fig. 1a). The flexibility of B-DNA allows several non-canonical conformations within the genome. Z-DNA was among the first secondary structures to be identified in dsDNA from circular dichroism studies and subsequently from crystallographic analysis (Wang et al. 1979; Ha et al. 2009) which revealed a left-handed helical structure with Watson-Crick base pairing (Fig. 1b). Unlike B-DNA where bases are in *anti*-conformation, Z-DNA shows alternating *syn-anti* conformation. This architecture gives the Z-DNA an elongated form, where the inter-base pair distance is longer than that

of B-DNA, coupled with a *zig-zag* arrangement of its sugar-phosphate backbone (Fig. 1b). The sheared base pair stacking seen in Z-DNA tends to pull bases more toward the outside of the helix and makes the structure more rigid than B-DNA (Fig. 1c and d). In addition, Z-DNA has no major groove.

An important non-canonical structure associated with Z-DNA is a BZ junction that forms where B-DNA and Z-DNA meet with an extrusion of bases at the junction between the B- and Z-DNA (Ha et al. 2005; Subramani et al. 2019). Accordingly, when Z-DNA forms in the genome, two BZ junctions flank each side of the Z-DNA-forming site (ZFS). Since Z-DNA formation is energy intensive, BZ junction formation is critical to alleviate torsional stress and stabilize the Z-DNA. Several studies have shown a clear sequence preference regarding the bases that can flip out at the junction, with A-T showing the highest propensity for extrusion (Bothe et al. 2012; Kim et al. 2018).

Conditions for Z-DNA formation

Z-DNA is a higher-order structure known to form in alternating purine-pyrimidine or pyrimidine-purine (APP) dinucleotide repeat sequences, with the propensity of Z-DNA to form in the order GC>CA>TA (Ellison et al. 1986; Ho et al. 1986; McLean et al. 1986). The formation of Z-DNA in vitro was initially tested under very high concentrations of NaCl. Additionally, several chemicals including spermine, spermidine, hexammine cobalt, and ruthenium complexes
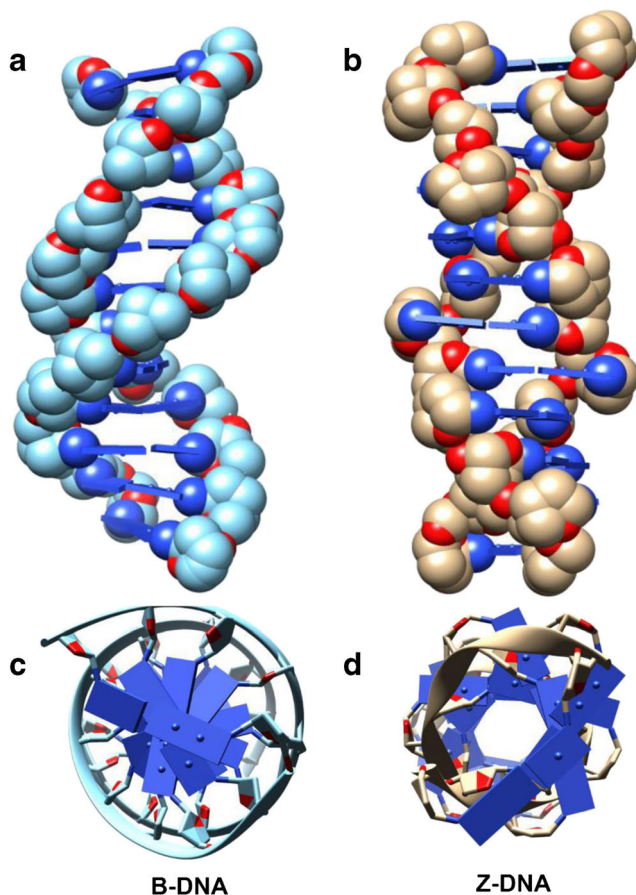
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Fig. 1 Comparison of B-DNA and Z-DNA structures. **a** B-DNA showing a right-handed helix. **b** Z-DNA showing left-handed helix and elongated structure. **c**, **d** Top view comparisons of B- and Z-DNA, respectively. B-DNA structure PDB ID: 1FQ2; Z-DNA structure PDB ID: 4LB5

are found to induce the conversion of B-DNA to Z-DNA (Subramani et al. 2019).

Some of the Z-DNA-forming conditions that are relevant in vivo are the presence of DNA supercoiling, Z-DNA-binding proteins, and base modifications. In early in vitro studies on plasmid DNA, it was found that negative supercoiling favors Z-DNA formation. Z to B transition could be facilitated by the addition of topoisomerases that can relax negatively supercoiled DNA. This is relevant in vivo because when transcription occurs, the movement of RNA polymerase II along the DNA strand generates positive supercoiling in front of, and negative supercoiling behind, the polymerase (Wang and Vasquez 2007). Z-DNA formation could possibly influence transcription by acting as a physical barrier for polymerase progression as seen in the case of prokaryotic systems (Peck and Wang 1985). In human cells, Z-DNA was found to form in actively transcribed regions of the genome and was confirmed using ChIP-Seq (Shin et al. 2016). Three hundred ninety-one ZFS were found in the human genome during this study. Chromatin remodeling can also affect the supercoiled state of the DNA and dynamically modulate Z-DNA formation. The

SWI/SNF or BAF complex of proteins can bind near the ZFS and induce changes in supercoiling, thereby favoring Z-DNA formation (Mulholland et al. 2012). Chromatin remodeling by BRG1 facilitates Z-DNA formation in the CSF1 gene promoter and regulates its expression (Liu et al. 2006). The interplay between chromatin remodeling and transcriptional induction of Z-DNA formation was observed in the case of BRG1-induced regulation of CSF1.

Z-DNA is induced and stabilized by Z-DNA-binding proteins (ZBPs) that directly interact with B-DNA. The E3L protein found in vaccinia viruses (Ha et al. 2004) and a PKR-like protein kinase (PKZ) found in fish (Kim et al. 2014) have been identified as ZBPs. Interestingly, two ZBPs found in mammals, adenosine deaminase acting on RNA 1 (ADAR1) and DNA-dependent activator of IFN-regulatory factors (DAI) (Pham et al. 2006; Wang and Vasquez 2007; Ha et al. 2008) have been identified and intensively studied. ZBPs have a conserved Z-DNA-binding domain (Zα) responsible for localization to Z-DNA. Both ADAR1 and DAI are interferon-inducible proteins that aid in nucleic acid clearance from the cytoplasm—an immune response against foreign DNA (Kim et al. 2011; Athanasiadis 2012). In vitro studies have reported that base modifications such as methylation of cytosines and guanines and deamination of adenine increase the propensity of Z-DNA stabilization (Wang et al. 1984; Xu et al. 2003). Like the effects of supercoiling, base modifications are also in vivo phenomena that act as means of epigenetic regulation. Similarly, histone acetylation also has the propensity to induce B- to Z-DNA transition (Zhang et al. 2016). Therefore, Z-DNA is a highly dynamic non-canonical DNA structure with several potent regulators.

Impact of Z-DNA formation in human disease

The tight regulation of Z-DNA formation within cells indicates that it plays an important role in cellular activities, such as in the recruitment of specific proteins/transcription activators or repressors, regulation of gene expression, control of genome instability, and eliciting immunogenic responses (Table 1). Specifically, in the case of humans, Z-DNA formation was associated with transcription of the *C-MYC* and corticotropin-releasing hormone (corticoliberin/*CRH*) genes, and in activation of the heme oxygenase-1 (*HO-1*) gene promoter (Wittig et al. 1992; Wolfl et al. 1996; Maruyama et al. 2013). The *ADAM-12* promoter contains a Z-DNA-forming sequence (ZFS) that negatively regulates ADAM-12 expression in normal cells (Ray et al. 2011). Loss of this element leads to overexpression of ADAM-12, which is typically a characteristic of many human cancers. It was found that MeCP2 can bind to the ZFS, recruit NF1 transcription factor to an adjacent site, and modulate *ADAM-12* repression. Lowered MeCP2 levels were found in metastatic breast cancer

Table 1 Roles of Z-DNA formation in the genome and its association with human diseases. While Z-DNA formation (red) is directly associated with diseases in the case of genome instability and immune responses, the association of Z-DNA directly in protein binding and transcription regulation is yet to be well established

Biological Function	Associated Gene	Related Disease	Reference
Recruiting Z-DNA Binding Proteins	MeCP2*	Breast Cancer	(Ray, Dhar et al. 2013)
	ADAR1	Aicardi-Goutières	(Rice, Kasher et al. 2012)
	ZBP/DAI	Influenza, Inflammation	(Sannula, Kuriakose et al. 2018)
	HIF1α*	Inflammation	(Bayele, Peyssonnaux et al. 2007)
	HO-1*	Emphysema	(Maruyama, Mimura et al. 2013)
Activation Repression Regulating Transcription	C-MYC	Cancer	(Wittig, Wölfl et al. 1992)
	CRH	Hormonal Imbalance	(Wolfl, Martinez et al. 1996)
	ADAM-12	Cancer	(Ray, Dhar et al. 2011)
Inducing Genome Instability	ETV6	Blood Cancer	(Wang and Vasquez 2007)
	Alu elements	Macular Degeneration	(Herbert 2019)
	CAG-Repeats	Huntington's Disorder, Spinocerebellar Ataxia	(Khan, Kolimi et al. 2015)
	BCL-2	Cancer	(Wang, Christensen et al. 2006)
	SCL	Cancer	(Wang, Christensen et al. 2006)
Eliciting Immune Responses		Systemic Lupus Erythematosus (SLE)	(Lafer, Valle et al. 1983)
		Amyotrophic Lateral Sclerosis (ALS)	(Allinquant, Malfoy et al. 1984)
		Crohn's Disease	(Allinquant, Malfoy et al. 1984)
		Polyradiculoneuritis	(Krishna, Fritzler et al. 1993)

*Protein known to bind to Z-DNA forming site, but the ability to bind to Z conformation is unknown

cell lines in addition to the loss of the ZFS, thereby underscoring the association of Z-DNA with human disease. Apart from the distinct role of Z-DNA in either activation or repression of gene expression, *HIF1α*-induced Z-DNA formation within a microsatellite in the *SLC11A1* promoter was shown to regulate its heritable variation and allele-specific expression (Bayele et al. 2007).

Z-DNA is known to induce structural instability by acting as sites for large-scale deletions in mammalian cells, especially in cancer-related genes such as *BCL2*, *C-MYC*, and *SCL* (Wang et al. 2006). Z-DNA sequences were also found to be enriched with several immunoglobin-related genes such as *ETV6* and associated with chromosomal translocations in these regions, especially in the case of blood-related cancers. ZFS overlap with recombination hotspots (Blaho and Wells 1989; Wahls et al. 1990) are also associated with retrotransposon activities and are found to be enriched in Alu elements (Herbert 2019). Z-DNA is also known to be immunogenic and has been implicated in systemic lupus erythematosus (SLE), Crohn's disease, polyradiculoneuritis, and amyotrophic lateral sclerosis (ALS), whose patients spontaneously produce anti-Z-DNA antibodies (Lafer et al. 1983; Allinquant et al. 1984). Interestingly, ZFS with (CCTG)n (CCAGG)n repeats also have a protective effect on DNA by reducing the potential slipped-strand DNA formation in the myotonic dystrophy type 2 (*DM2*) gene (Edwards et al. 2009).

In addition, BZ junctions are speculated to be sites for CAG trinucleotide repeat-induced instability (Khan et al. 2015). Similarly, CGG repeats associated with a fragile X chromosome and GAC repeats associated with skeletal dysplasia can also form Z-DNA (Vorlíčková et al. 2001; Renciuk et al. 2010). There is also strong evidence of Z-DNA formation in the hippocampus of Alzheimer's patients, implicating Z-DNA in neurodegenerative disorders (Suram et al. 2002).

Future perspectives

Z-DNA is a dynamic non-canonical DNA found within the human genome, whose formation is influenced by several molecular players. The multifarious roles that Z-DNA play in human disease present an immense opportunity to target Z-DNA by chemical modulators from either natural or synthetic sources. Therapeutic strategies using small molecules targeted against Z-DNA would theoretically have lower non-target effects due to their conformational specificity. Particularly of intrigue are the BZ junctions whose formation and role have yet to be well studied. It is possible that the extruded bases can be targeted by DNA repair enzymes, transcription factors, or small molecules. A significant opportunity lies in the discovery of novel Z-DNA inducing and repressing small molecules because the current Z-DNA modulators are unsuitable for therapeutic use or have failed in

clinical trials (Fuertes et al. 2006). Hence, there is immediate need and urgency to conceptualize and design screening strategies to look for novel modulators of Z-DNA—BZ inducers, Z binders and Z disruptors, ZB inducers, and BZ inhibitors. The future, therefore, holds many exciting opportunities to further our understanding of Z-DNA formation and its targeting for therapeutic purposes.

Compliance with ethical standards

Conflict of interest Subramaniyam Ravichandran declares that he has no conflict of interest. Vinod Kumar Subramani declares that he has no conflict of interest. Kyeong Kyu Kim declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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