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Review

When secondary comes first – The importance of non-canonical DNA structures

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ABSTRACT

Secondary structure-forming DNA motifs have achieved infamy because of their association with a variety of human diseases and cancer. The 3rd FASEB summer conference on dynamic DNA structures focused on the mechanisms responsible for the instabilities inherent to repetitive DNA and presented many exciting and novel aspects related to the metabolism of secondary structures. In addition, the meeting encompassed talks and posters on the dynamic structures that are generated during DNA metabolism including nicked DNA, Holliday junctions and RNA:DNA hybrids. New approaches for analysis and sequencing technologies put forth secondary structures and other DNA intermediates as vital regulators of a variety of cellular processes that contribute to evolution, polymorphisms and diseases.

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1. Introduction

This year is the 50th anniversary of the discovery of the G4 quartet. The 2012 FASEB Summer Research Conference on Dynamic DNA Structures in Biology commemorated this event by dedicating a large fraction of the meeting to the subject. Talks ranged from Martin Gellert's (NIH) first hand account of how studies of a jelly-like substance formed by guanylic acid led to the identification of G-quadruplexes in 1962 [1] to more recent work demonstrating the functional significance of such structures *in vivo*. In addition, this meeting, organized by Nancy Maizels (University of Washington) and Sergei Mirkin (Tufts University) at Saxtons River, VT, June 17–22, covered a wide variety of other alternate DNA structures including triplexes, hairpins, cruciforms, RNA:DNA hybrids and Holliday junctions and their roles in the metabolism of prokaryotic and eukaryotic cells. Through a series of talks and poster sessions, the meeting emphasized that unusual DNA secondary structures are widespread in all living organisms where they have profound effects on replication, transcription and genome stability. Some of these effects are positive, affecting normal development and the generation of genetic diversity, whilst other effects are negative and result in a variety of genetic disorders and cancer in humans (Fig. 1).

While much has been learned about the behavior of many of these structures, our understanding of their incidence in the genome is still incomplete. As was highlighted in the keynote address by Jeffrey Strathern (NCI), the inability of some of these DNA motifs to be propagated in *Escherichia coli* (e.g. long palindromes) and to be amplified or sequenced has resulted in their underrepresentation in whole-genome sequencing analyses of complex genomes including humans. However, as was accentuated in the keynote address and other talks in the meeting, the field is entering an exciting new era where more accurate sequencing technologies and bioinformatics tools for analysis of these sequences are emerging.

Below we briefly describe these topics as well as other new findings discussed at this meeting that contribute to our understanding of the dynamic nature of DNA.

2. G-quartets – the birthday boy

G-rich DNA molecules can form inter- or intra-molecular hydrogen bonds to form square planar arrays of 4 guanines known as G-quartets. A series of G-quartets results in a quadruplex structure frequently referred to as a G-quadruplex, G-tetraplex or G4-DNA. The guanines in the quadruplex are held by non-Watson–Crick hydrogen bonds, termed Hoogsteen base-pairs. The topology of the quadruplex varies depending on the orientation of the DNA strands involved, the length of the G-rich region and its nucleotide composition. The consensus sequence commonly used in genome-wide bioinformatic studies to identify G-quartets is d(G3 + N1–7)4

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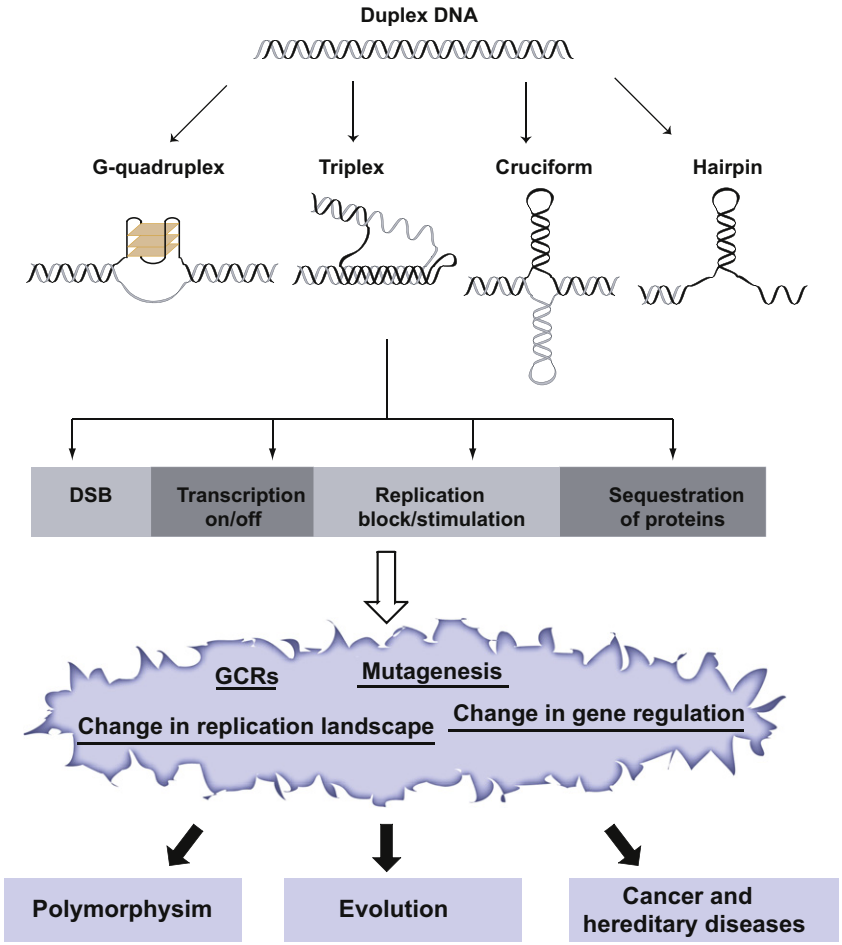

Fig. 1. The role of secondary structure-forming repeats in genome function and integrity. As was evident from the meeting, the formation of secondary structures or the presence of the sequence motif *per se* can lead to double strand breaks (G-quartets, triplexes, cruciforms and hairpins); induction (G-quartets and GAA/TTC tracts) or inhibition of transcription (G-quartets and triplexes); initiation (triplexes) or stalling of replication (G-quartets, triplexes, cruciforms and hairpins); and sequestration of cellular proteins (G-quartets, triplexes and r(CUG) hairpins). These processes likely contribute to the generation of polymorphisms, to genome evolution and to a variety of diseases via gross-chromosomal rearrangements, mutagenesis, dysregulation of gene expression and alteration of the replication landscape.

[2]. However, as was pointed out by Jean-Louis Mergny (IECB), experimental studies demonstrate that this pattern is not perfect and yields many false-positives and false-negatives. For instance, it does not predict human mini-satellite CEB25 to be a G4-forming motif although structural analysis proves it to be so [3]. Mergny's laboratory has addressed this conundrum by looking for G clusters in the genome which have pronounced GC asymmetry. The new and improved algorithm for detection of G-quadruplex forming motifs also analyzes large genomic regions to take into account the observation that G4-DNA might contain interstitial loops and imperfections that still allow formation of stable secondary-structure.

Kyle Miller (Currently at University of Texas at Austin) presented a study carried out in the labs of Shankar Balasubramanian and Steve Jackson (University of Cambridge). He used pyridostatin (PDS), a small molecule that interacts with G-quadruplexes, to identify G4-forming motifs in the human genome. The loci for PDS binding colocalized with binding sites for Pif1, a known G4-DNA-unwinding helicase. The PDS-mediated stabilization of the G-quadruplex causes double strand breaks (DSBs), allowing the G-quadruplex forming region to be identified by using chromatin immunoprecipitation of γH2AX followed by next-generation sequencing. Twenty-five cancer related genes, including several known oncogenes, were identified including *SRC*. Interestingly, oncogene expression was down-regulated upon treatment with PDS, suggesting the potential for the use of small molecules that interact with secondary structures as therapeutic agents. The phenomenon of oncogene down-regulation by G-quadruplex stabilizing agents is likely due to the fact that G4-DNA can effectively block the transcription machinery. In general, G4-DNA has been shown to be enriched in gene regulatory regions with preponderance for promoter regions in both prokaryotes and eukaryotes (Reviewed in Refs. [2,4]).

A very clear demonstration of the role of G4 DNA was provided by Laty A. Cahoon (Northwestern University, H. Steven Seifert's laboratory) who showed that transcription across a G-rich region upstream of the pilE locus of *Neisseria gonorrhoeae* leads to G4-DNA formation. This pilE G4 DNA structure results in nicks that promote recombination and thus pilin antigenic variation that enables this microbe to evade the host's immune response.

A G-quadruplex can also present an obstacle for the DNA replication machinery and trigger genomic instability. Alain Nicolas and Aurele Piazza, a graduate student from his laboratory (Curie Institute) studied metabolism of the G-quadruplex-forming human mini-satellites CEB1 and CEB25 in the yeast *Saccharomyces cerevisiae*. Interestingly, these 2 mini-satellites behave differently *in vivo*. Unlike CEB25, CEB1 was found to be extremely prone to size variations when the G-rich strand is a template for leading strand

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