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Review paper

Attachment of Small Molecule with DNA and RNA

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ABSTRACT

The four natural DNA bases A-Adenosine, T-Thymine, G-Guanine, C-Cytosine are associate in base pairs as (A=T and G≡C), allowing the attached DNA strands to assemble into the canonical double helix of DNA which is duplex of DNA, also known as $\mathcal{B}$-DNA. The intrinsic supra molecular properties of nucleo bases make other associations possible such as base triplets or quartets, which thus translates into a diversity of DNA structures is ripe with approximately 20 letters, (from A- to Z-DNA); however, only a few of them are being considered as key players in cell biology and by extension, valuable targets for chemical biology invention. In the present review, we summarise (1) what is known about alternative DNA structures (2) what are they? (3) When, where and how they fold?

These are all proceeded to discuss further and those considered nowadays as valuable therapeutic targets. We discuss in more detail the molecular tools (ligands) that have been recently developed to target these structures. In order to intervene in the biological processes, particularly three and four ways of DNA junctions are involved there. This new and simulating chemical biology playground allows for devising innovative strategies to fight against genetic diseases.

1. Introduction

The primary function of RNA is to create proteins via translation. RNA carries genetic information that is translated by ribosome into various proteins necessary for cellular processes. mRNA, rRNA, and tRNA are the three main types of RNA involved in protein synthesis mRNA (messenger RNA): it provides the template for protein synthesis during translation. tRNA (transfer RNA): it brings amino acids and reads the genetic code during translation. rRNA (ribosomal RNA): it plays a structural and catalytic role during translation.

The four natural RNA bases A-Adenosine ,U-Uracil, G-Guanine, C-Cytosine are associate in base pairs as (A=U and G≡C), allowing the attached DNA strands to assemble into the canonical double helix of RNA which is duplex of RNA, also known as $\mathcal{B}$-RNA. The intrinsic supra molecular properties of nucleo bases make other associations possible such as base triplets or quartets, which thus translates into a diversity of RNA structures is ripe with approximately 20 letters, (from A- to Z-RNA); however, only a few of them are being considered as key players in cell biology and by extension, valuable targets for chemical biology invention. Uracil is replaced by Thymine in case of DNA. In the present review, we summarise (1) what is known about alternative RNA structures (2) what are they? (3)When, where and how they fold?

These are all proceeded to discuss further and those considered nowadays as valuable therapeutic targets. We discuss in more detail the molecular tools (ligands) that have been recently developed to target

these structures. In order to intervene in the biological processes, particularly three and four ways of RNA junctions are involved there. This new and simulating chemical biology playground allows for devising innovative strategies to fight against genetic diseases.

RNA is crucial for gene expression and regulation. Recent advances in understanding of RNA biochemistry, structure and molecular biology have revealed the importance of RNA structure in cellular processes and diseases. Various approaches to discovering drug-like small molecules that target RNA structure have been developed. This review provides a brief introduction to RNA structural biology and how RNA structures function as disease regulators. We summarize approaches to targeting RNA with small molecules and highlight their advantages, shortcomings and therapeutic potential. RNA importance is increasingly appreciated as regulators of human diseases and consequently as potential drug targets. Novel methods to target RNA structure with small molecules rather than RNA sequence have been developed. Knowledge and principles of RNA structure are highly desired to develop more effective RNA structure-targeting drugs.

The four natural DNA bases A-Adenosine ,T-Thymine, G-Guanine, C-Cytosine are associate in base pairs as (A=T and G≡C), allowing the attached DNA strands to assemble into the canonical double helix of DNA which is duplex of DNA, also known as $\mathcal{B}$-DNA. The intrinsic supra molecular properties of nucleo bases make other associations possible such as base triplets or quartets, which thus translates into a diversity of DNA structures is ripe with approximately 20 letters, (from A- to Z-DNA); however, only a few of them are being considered as key players in cell biology and by extension, valuable targets for chemical biology invention. In the present review, we summarise (1) what is known about alternative DNA structures (2) what are they? (3)When, where and how they fold?

These are all proceeded to discuss further and those considered nowadays as valuable therapeutic targets. We discuss in more detail the molecular tools (ligands) that have been recently developed to target these structures. In order to intervene in the biological processes, particularly three and four ways of DNA junctions are involved there. This new and simulating chemical biology playground allows for devising innovative strategies to fight against genetic diseases.

The DNA alphabet is naturally restricted to four letters, i.e. A for adenine, C for cytosine, G for guanine and T for thymine (1,2) (although some creative scientists have achieved the juggling act to expand it to 6 in living semi synthetic organisms (3,4), and then 8 (5), mimicking what was discovered in bacterio phages >4 decades ago) (6–9). In contrast, the DNA structure alphabet is far richer, with >20 letters used to date as descriptors of secondary structures). The canonical, so called Watson–Crick, structure is referred to as B-DNA (as the X-ray crystallographic structure was obtained by Rosalind Franklin after hydration of a first sample ('A') of high-quality DNA provided by Rudolf categorized as a non-B-DNA structure, spanning from A-DNA (thus, dehydrated duplex) (11) to Z-DNA (duplex of inverted helicity, Z for zigzag) (10,15).

The central dogma of biology, heralded by Francis Crick in 1957 (16,17), has placed the B-DNA at the very Centre of all molecular biology efforts invested, and discoveries made, after the elucidation of its double helix structure (2,13,18). This has kept the limelight away from reports published in succeeding years on the ability of DNA to fold into a variety of non-B-DNA structures (Figure 1), including: the triple helix (or triplex (19), termed H-DNA given its homo purine (hPu)/homo pyrimidine (hPy) nature, also refer- ring to hinged DNA) (20), first identified in RNA in 1957(21) before being characterized in DNA in 1979; (22) the G-quartet in 1962 (23), the constitutive unit of the quadruple helix G-quadruple (G4-DNA, or G-DNA) whose formation was demonstrated in 1988; (24) the tetra-stranded four-way DNA junction proposed as a model to explain gene conversion in 1964 by Robin Holliday (consequently called the Holliday junction) (25), predicted in 1966 (26) and demonstrated in vitro (and termed cruciform DNA, C- DNA) in the early 80s; (27,28) the Z-DNA first detected in 1967 (as a B-DNA of inverted, left-handed helicity) (29) be- fore being firmly confirmed in 1979 (30), etc.

2. Literature Review

2.1 Methodology in terms of Systematic Review and Literature Review in broad aspect

As per the above available primary data of the research I have searched DNA/RNA Structure in the Mendeley search Engine, here I have found more than 99000 papers. To minimize and to get best result I have changed the searched words into various option, where only journal papers and Thesis from year 2020 to 2024 , which give me best results and was shorten the work into nearly 29032 papers in that searching date25/11/2023. Out of this finding the attachments of small molecules shorten to nearly 7292 open access journal papers papers which shorten to m I have made a link of nearly 200 papers in the Mendely Reference Management Software where a prior registration with my email account was done. After that I have made an Excel Sheet where searching tools, paper name, Authors, Abstracts, volume, issue date, Key words, URL, Conclusion etc. data were tabulated. Apart from that Departmental library/

central Library books also help me in this review work. But systematic review is done with the help of the mentioned software. It is true the available data are different in different date and time. To get best result it should be carried out in the same date with searching tools and words etc. Some basic steps has been followed to carry out this systematic review of this available primary data on the taken topic and basic ideas.

First in this systematic review process I have searched on the topic of (a) coral reef zone in India. (b) The cause of bleaching process there. With the help of various searching engine I have found the best available papers or book on it. Here the following process has been described in the term of systematic Review. Total no of shorted papers in the excel sheet is 200.

Different Searching results: (14)

(1) Screening: From Scopus-Elsevier searching using key words as "DNA/RNA structure, searching date 25/11/2022, total findings was 29038 for at any time, since 2020 total findings minimizes as7292, where only open access journals.

(2) Screening: small molecules attachments Elsevier.com results nearly 200, changing key words "attachments with small molecules with DNA/RNA" results only 350 available for open access papers.

(3) 3rd Screening: Changing date 233/12/2023 time 11.35A.M using key words" coral bleaching and India" from Elsevier, all results as 200 where Web page: 73, Books: 45, Journals: 102, thesis: remaining

(4) **4th Screening:** Changing key words as in the same Elsevier in the same date "cloning of DNA, results nearly same

(5) **5th Screening**: Changing key words as in the published journal: 200,

(6) **Screening:** Changing the key words DNA replication also going through abstract suggested papers result same.

Systematic Review is done in the Excel Sheet, where the 56 papers data are inputted in tabular form. Some of papers which has been carried out there are representing in tabular form in word format in below. The data in tabular form helps us to get best results on research work progress on the above topic. Various graphical representation or charts could be drawn. And from Results conclusion could be drawn.

In the Excel sheet we input the following data in Table 1:

Item Type-SL. No
Publication Year
Search Engine
Journal or Book name
Search Strings
Author Name
Title
Publication Title
Issue
Volume
Abstract
Key Words
Methodology
Sample Size
STUDY Area
Result
Conclusion
Paper Publication
Citation

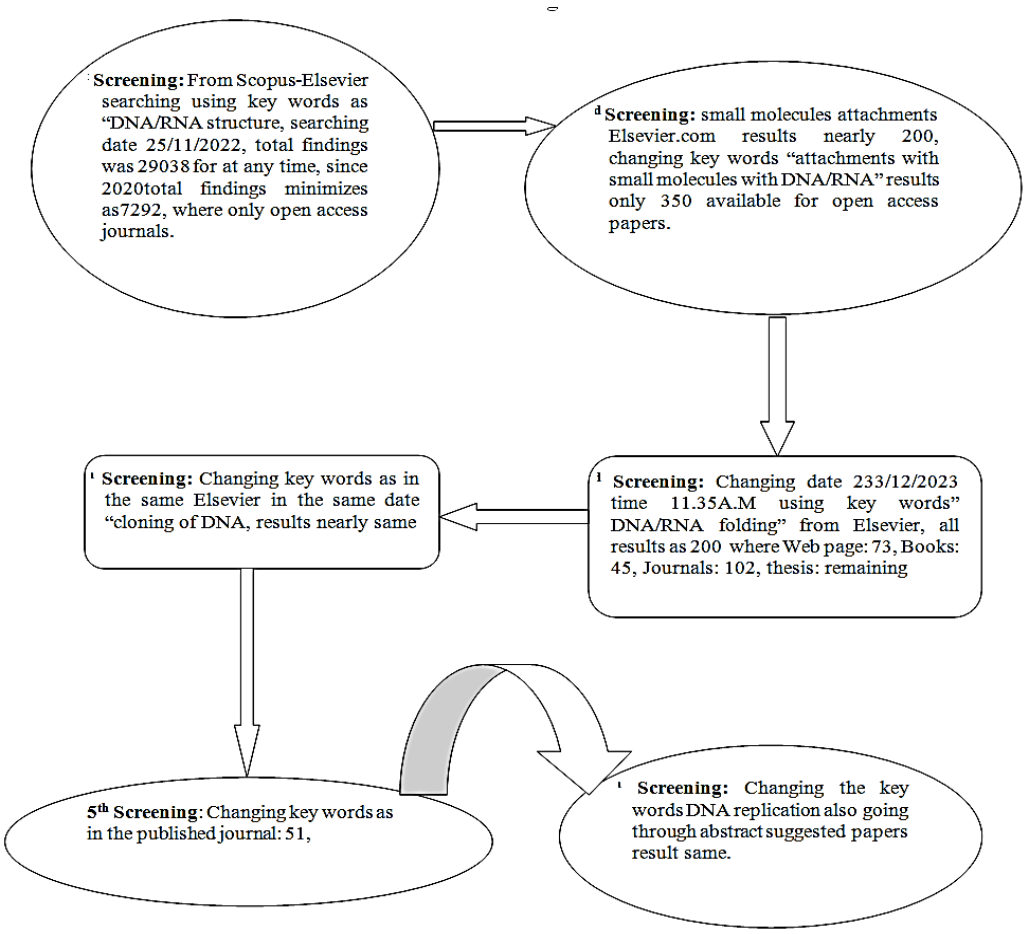
For example referred from Appendix the carried out systematic review in the form of Excel sheet, comes in the following word form in Table 2:

Item Type-1	**Journal Articles**
Publication Year	2022
Search Engine	Mendeley.
Journal or Book name.	Scopus, Science Direct, Pubmade etc
Search Strings	DNA/RNA structure.
Author Name	Goronzy, Isabel N., Quinodoz, Sofia A., Jachowicz, Joanna W., Ollikainen, Noah, Bhat, Prashant, Guttman, Mitchell
Title	Simultaneous mapping of 3D structure and nascent RNAs argues against nuclear compartments that preclude transcription
Publication Title	Simultaneous mapping of 3D structure and nascent RNAs argues against nuclear compartments that preclude transcription
Issue	9
Volume	41
Abstract	Mammalian genomes are organized into three-dimensional DNA structures called A/B compartments that are associated with transcriptional activity/inactivity. However, whether these structures are simply correlated with gene expression or are permissive/impermissible to transcription has remained largely unknown because we lack methods to measure DNA organization and transcription simultaneously. Recently, we developed RNA & DNA (RD)-SPRITE, which enables genome-wide measurements of the spatial organization of RNA and DNA. Here we show that RD-SPRITE measures genomic structure surrounding nascent pre-mRNAs and maps their spatial contacts. We find that transcription occurs within B compartments—with multiple active genes simultaneously co localizing within the same B compartment—and at genes proximal to nucleoli. These results suggest that localization near or within nuclear structures thought to be inactive do not preclude transcription and that active transcription can occur throughout the nucleus. In general, we anticipate RD-

	SPRITE will be a powerful tool for exploring relationships between genome structure and transcription.
Key Words	DNA Structure, A/B Compartment, genes. RD-SPRITE.
Methodology	3D structure
Sample Size	*Large*
STUDY Area	*DNA/RNA Structural area*
Result	This work attempts to Study the link between structural studies and modification by auto docking in Vina software.
Conclusion	These results suggest that localization near or within nuclear structures thought to be inactive do not preclude transcription and that active transcription can occur throughout the nucleus. In general, we anticipate RD-SPRITE will be a powerful tool for exploring relationships between genome structure and transcription.
Paper Publication	Yes, International
Citation	https://doi.org/10.1016/j.celrep.2022.111730

3. Result Discussion

Flowchart of the screened articles in chart in Figure 1



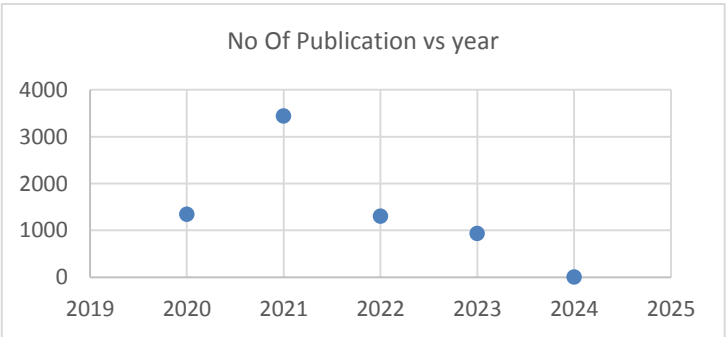
Systematic review work for bibliography/ references has done as per training and exposures gained from International exposures from IIP- International workshops and seminar, arranged by Eudoxia research centre India, and Eudoxia Research University, USA

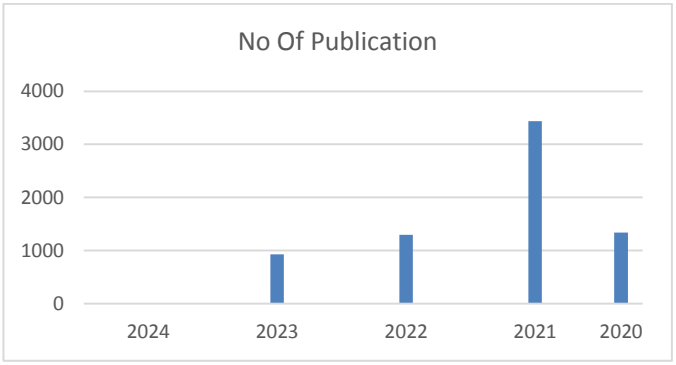
From Excel sheet of systematic Review, applying filters give this following Table 3:

Year	No of Publication
2024	2
2023	931
2021	1299
2020	1341

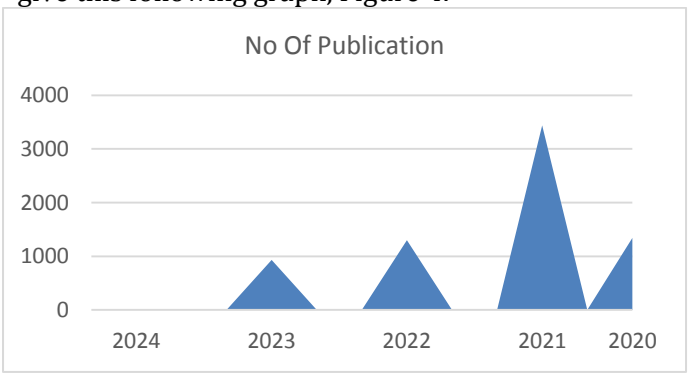
Putting into Bar diagram results shows as follows in graph-1,2 Figure-2,3.

:





From Excel sheet in the Appendices of systematic Review, applying filters give this following graph, Figure 4.



Literature review shows the work of attachements of small molecules with DNA/RNA could be a great work in terms of paper publication and other Noble works in this Analytica Chemistry area in this incoming years with huge job prospects in India and USA .

Understanding of the Research Gaps

- Understanding of the structures of ATGC,U.
- Understanding of the DNA/RNA folding 3D structures.
- Molecular hybridization concept or Host guest mechanism A+B≡C, or molecular docking, protein ligand mechanism understanding.
- Understanding of n- folding
- Understanding of cloning.
- Drug design strategy.
- or protein repairing for cloning purposes.

4. Research Methodology

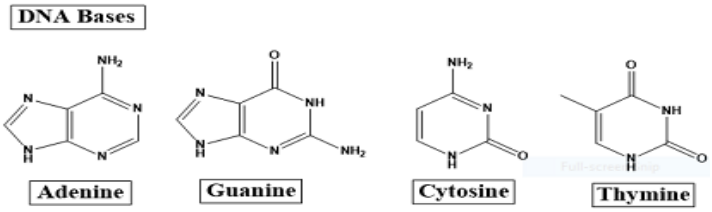
4.1 DNA/RNA bases

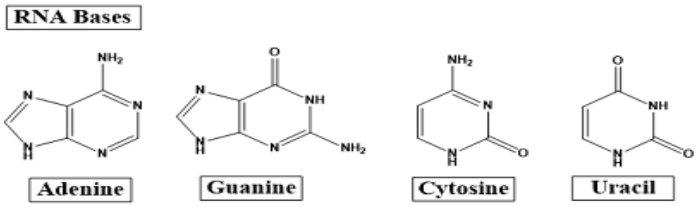
- A-Adenosine ,
- T-Thymine or U-Uracil,
- G-Guanine,
- C-Cytosine

All are associate in base pairs as (A=U and G≡C) in RNA. In case of DNA, Thymine=T is present instead of Uracil. All are associate in base pairs as (A=T and G≡C) in DNA.

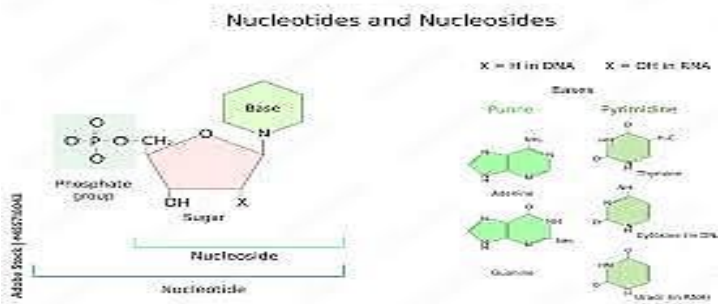
4.2 Topological diversity of DNA stems from supramolecular chemistry considerations

Nature of binding is here H-bonding (A=U and G≡C) Nuclei bases (A, C, G, T) associate through the formation of hydrogen bonds (H-bonds), two in the A T base pair, three in the G C base pair, allowing for a dynamic assembly/disassembly without substantial energy penalty.

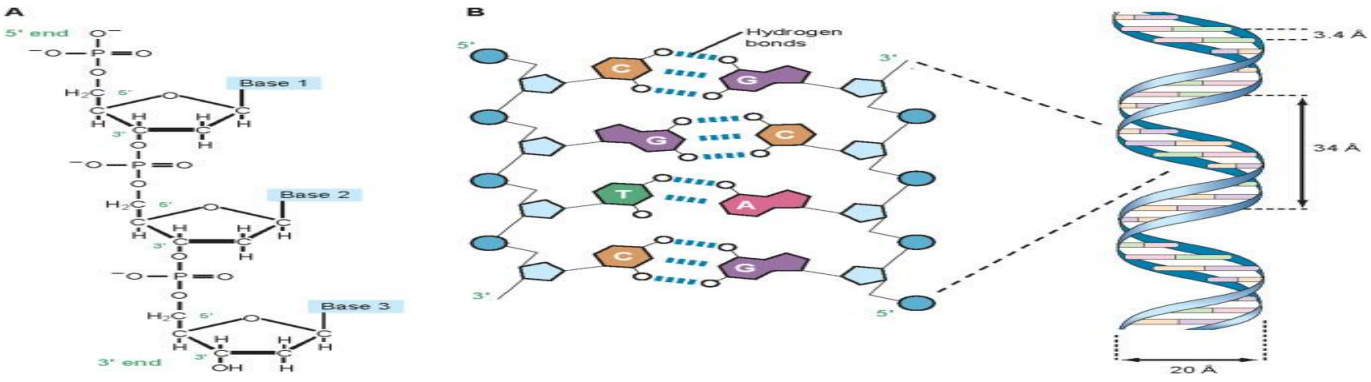


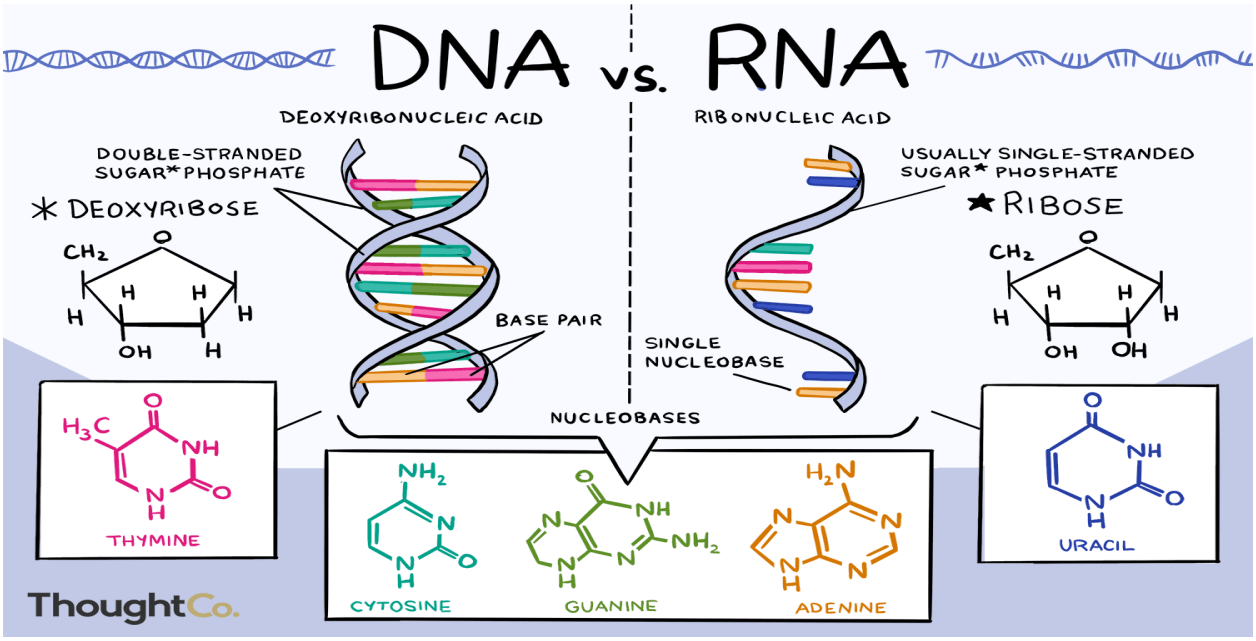


4.3 Structures of DNA/RNA Bases (ATGC/AUGC)



Details of H-bonding in DNA/RNA represented simply as (A=T, G≡C.) below:





4.4 Replication (A Theoretical Methodology Discussion)

This is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules. DNA replication is one of the most basic processes that occur within a cell.

What are the 4 steps of replication?(Figure-5)

- Step 1: Replication Fork Formation. Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. ...
- Step 2: Primer Binding. The leading strand is the simplest to replicate. ...
- Step 3: Elongation. ...
- Step 4: Termination

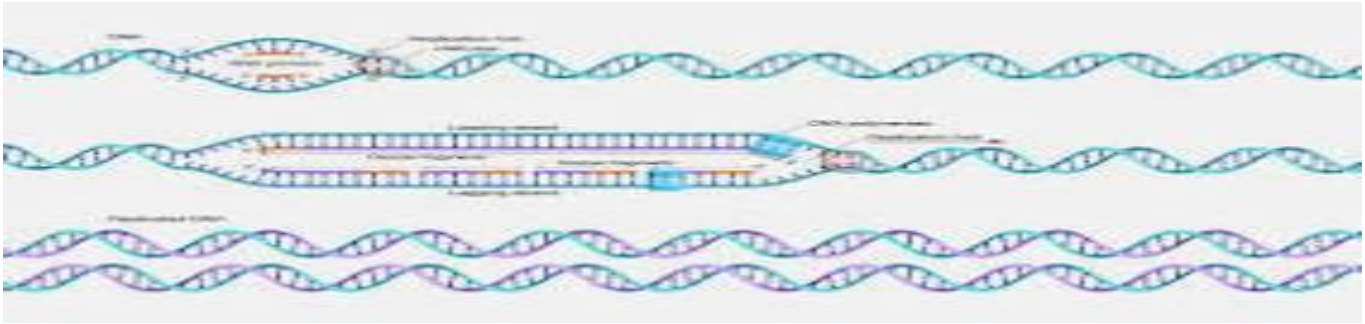


Figure-6,DNA REPLICATION PROCESS

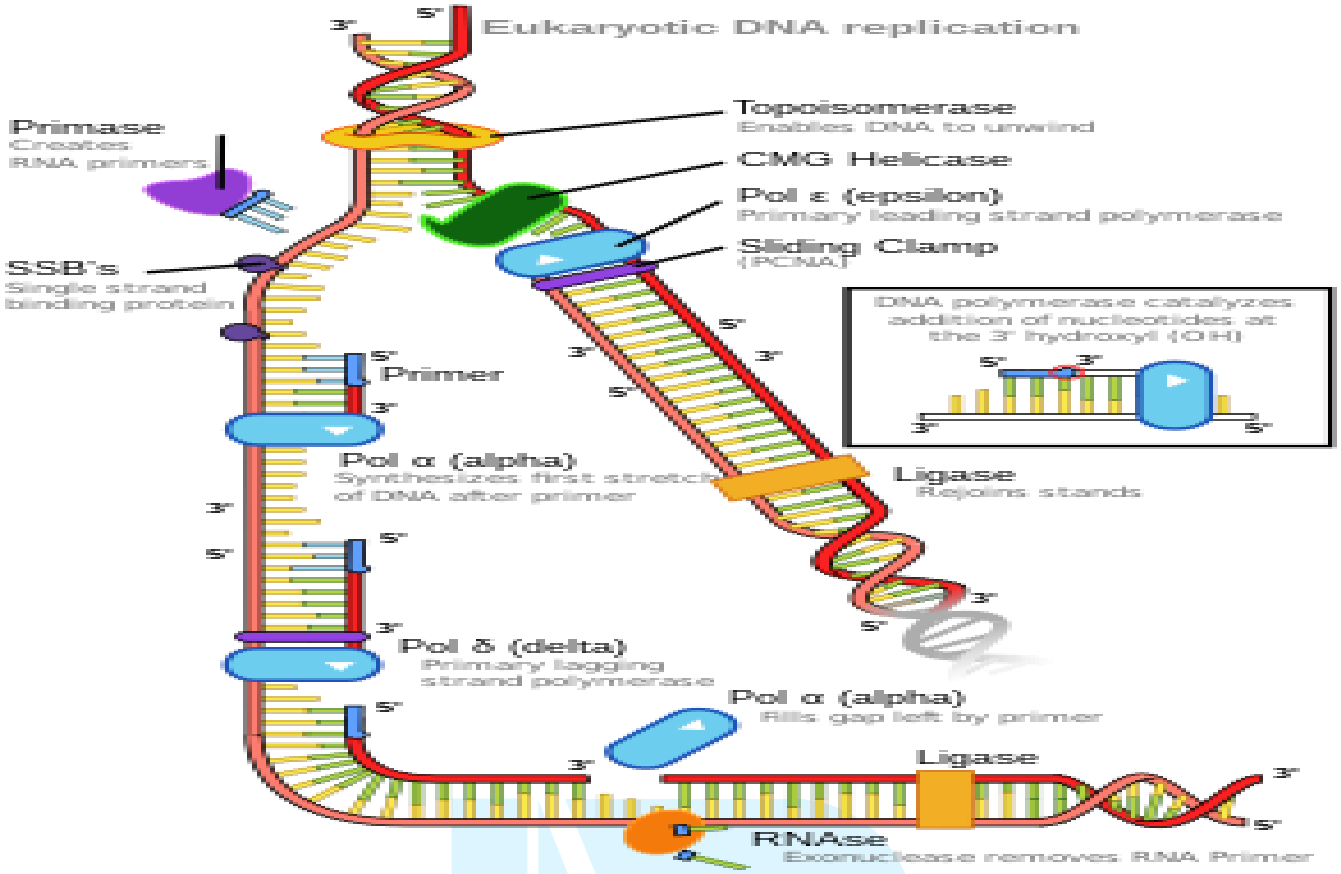
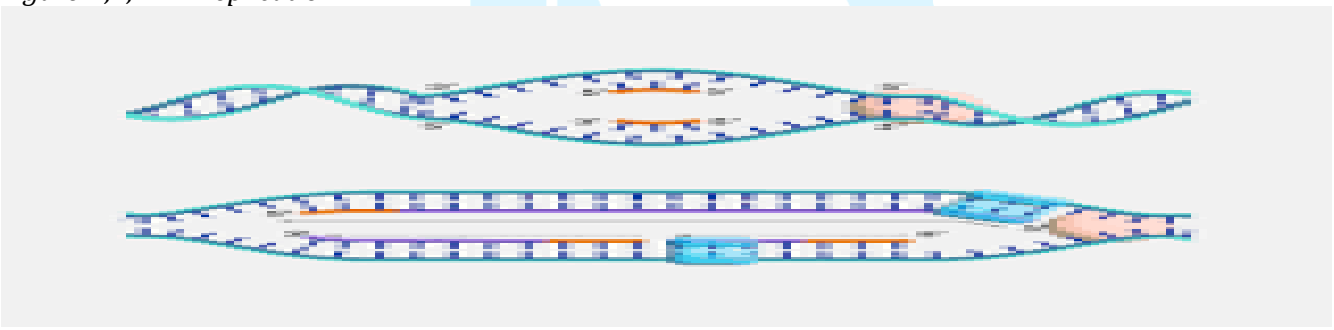
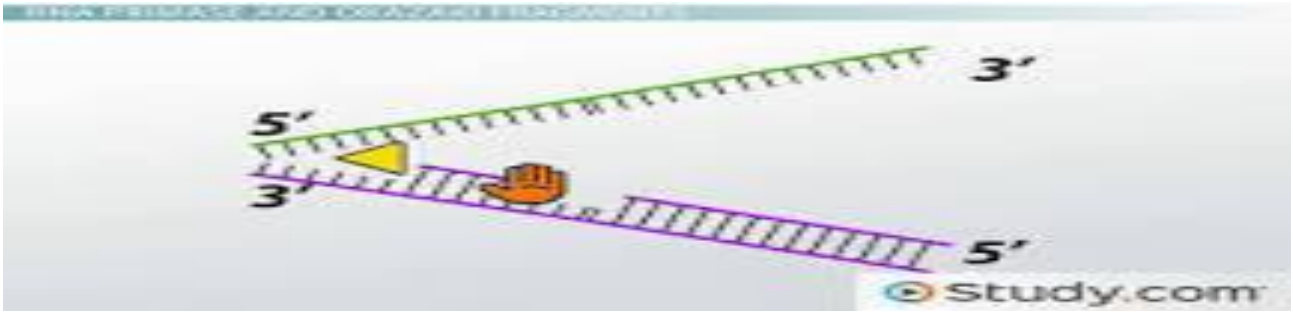


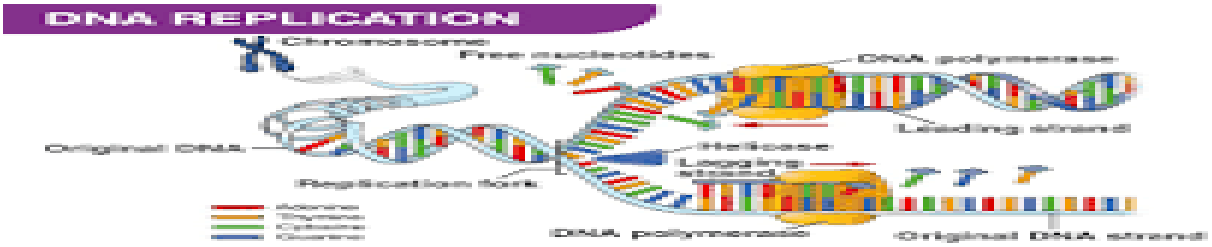
Figure-7,8;DNA Replication



The complete process of DNA Replication involves the following steps:

- Recognition of initiation point. End nuclease enzyme.
- Unwinding of DNA.
- Template DNA.
- RNA Primer.
- Chain Elongation.
- Replication forks. Okazaki fragments continuous strand. Discontinuous strand. ...
- Proof reading.
- Removal of RNA primer and completion of DNA strand.(figure-9,10 replication steps)





The four main enzymes involved in DNA replication are DNA helices', RNA primase, DNA polymerase, and DNA ligase. These enzymes work together to open up the DNA strand in replication bubbles and copy the DNA strands semi-conservatively

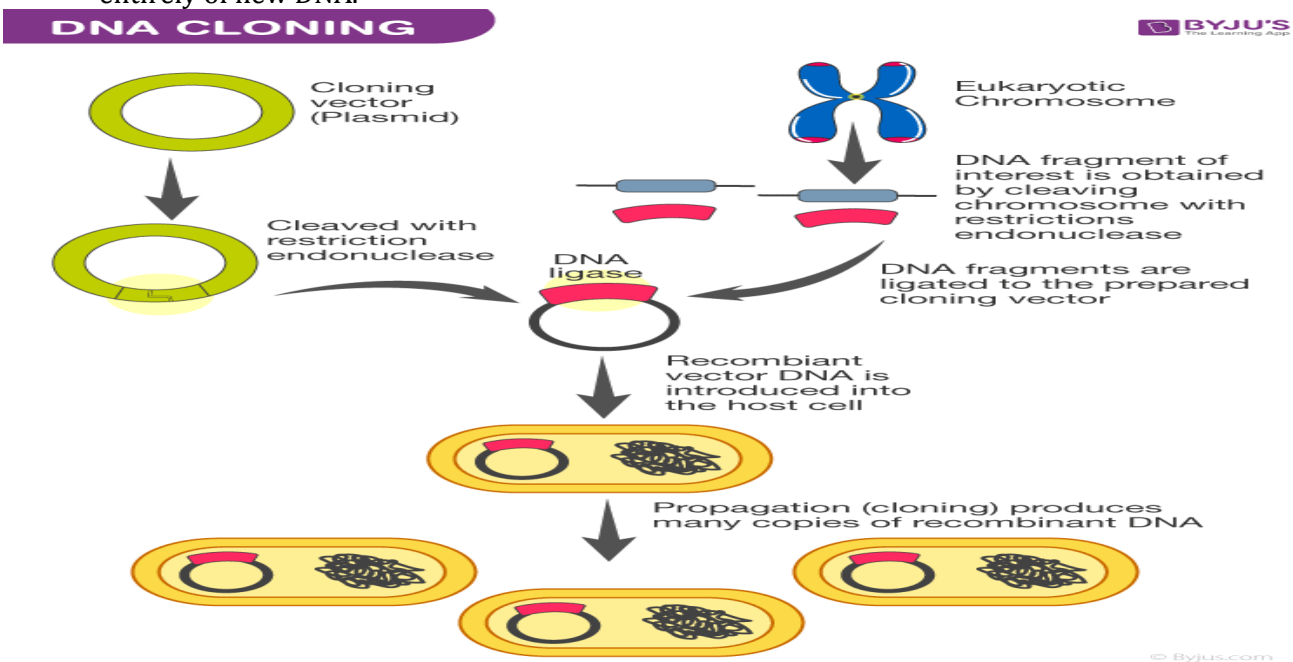
What are the 5 types of DNA replication?

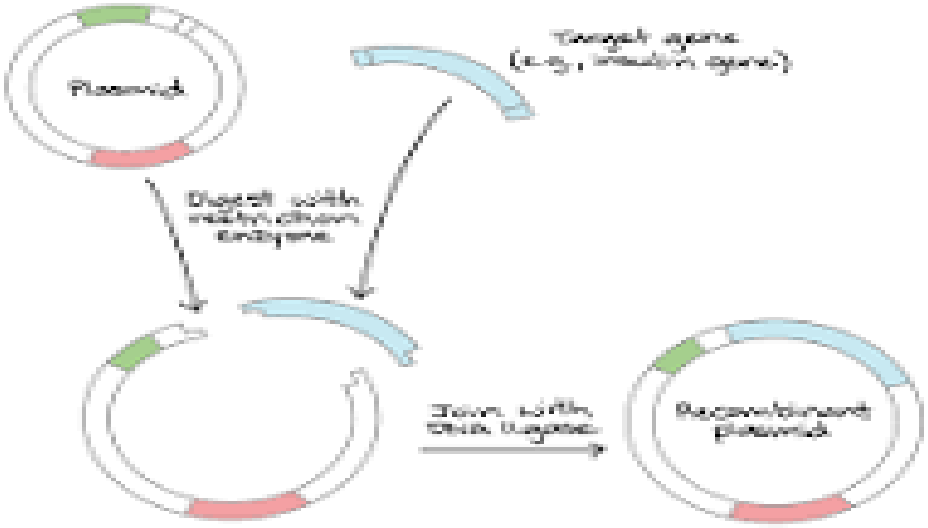
The three models for DNA replication

- Conservative. Replication produces one helix made entirely of old DNA and one helix made entirely of new DNA.
- Semi-conservative. Replication produces two helices that contain one old and one new DNA strand.
- Dispersive.
- What is an example of DNA replication?

Replication is the process by which a cell copies its DNA prior to division. In humans, for example, each parent cell must copy its entire six billion base pairs of DNA before undergoing mitosis.

D.4. DNA CLONING (Figure-11)





What are the 4 steps in cloning? (A Theoretical Methodology Discussion):

In the classical restriction enzyme digestion and ligation cloning protocols, cloning of any DNA fragment essentially involves four steps:

- isolation of the DNA of interest (or target DNA),
- ligation,
- Transfect ion (or transformation), and.
- a screening/selection procedure
- What is cloning method?
- Cloning is a technique scientists use to make exact genetic copies of living things. Genes, cells, tissues, and even whole animals can all be cloned. Some clones already exist in nature.

Uses of DNA Clone

In basic research labs, biologists often use DNA cloning to build artificial, recombinant versions of genes that help them understand how normal genes in an organism function.

Why use PCR for cloning?

PCR cloning is a rapid method for cloning genes, and is often used for projects that require higher throughput than traditional cloning methods can accommodate. It allows for the cloning of DNA fragments that are not available in large amounts.

Innovative Objectives on Folding and Attachments of DNA/RNA

Changing targeting molecules we can enhance or decrease the folding types or pattern.

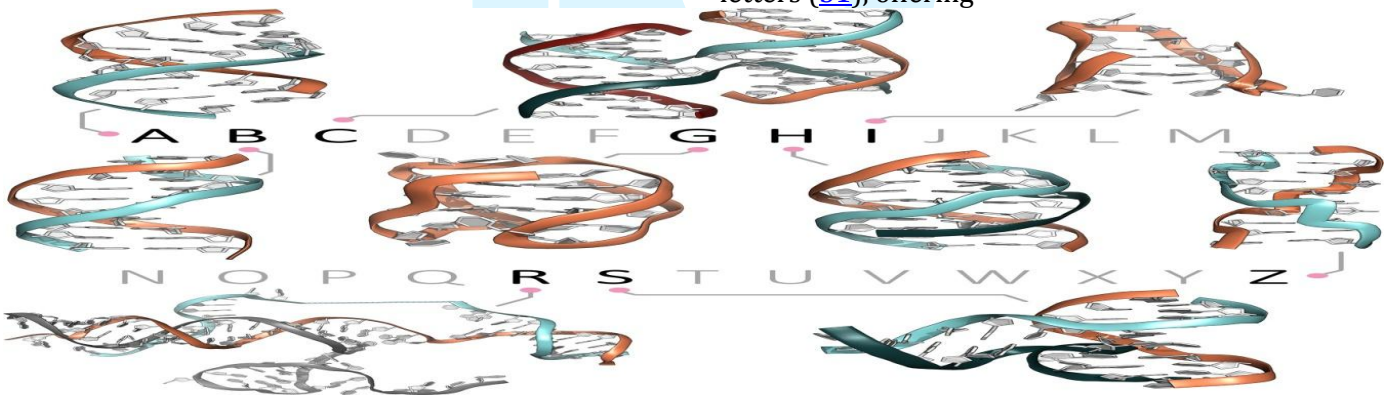


Figure-12(H Bonding in DNA)

The canonical, so called Watson–Crick, structure is referred to as B-DNA (as the X-ray crystallographic structure was obtained by Rosalind Franklin after hydration of a first sample ('A') of high-quality DNA provided by Rudolf categorized as a non-B-DNA structure, spanning from A-DNA (thus, dehydrated duplex) (11) to Z-DNA (duplex of inverted helicity, Z for zigzag) (10,15).

The central dogma of biology, heralded by Francis Crick in 1957 (16,17), has placed the B-DNA at the very Centre of all molecular biology efforts invested,

Present studies predicts that as per stability of the molecules, transition states and activation energy KCP (Kinetically Controlled Product) or TCP (Thermodynamically Controlled Product)find there routs.

Changing solvent/temperature may also effect on structure folding.

We have to synthesise those structures those are useful tools for drug design or protein repairing for cloning purposes.

Update of Research design by software

- Molecular docking tools like CDHIT, Auto dock Vina in the work of Bioinformatics
- Use of Protein /Ligand DATA Bank (PDB software), Avogadro, PubChem etc.
- chemspider in the chemical drawing and structural analysis
- Use of AI-Artificial Drug design Intelligence for drug design and other.

5. Result and Discussion

The topological diversity of DNA stems from supra molecular chemistry considerations: nucleo bases (A, C, G, T) associate through the formation of hydrogen bonds (H-bonds), two in the A T base pair, three in the G C base pair, allowing for a dynamic assembly/disassembly without substantial energy penalty. However, nucleo bases are not exclusive in their H-bond- mediated association, and >20 different pairing modes are possible involving two of the four letters (31), offering

and discoveries made, after the elucidation of its double helix structure (2,13,18). This has kept the limelight away from reports published in succeeding years on the ability of DNA to fold into a variety of non-B-DNA structures (Figure 1), including: the triple helix (or triplex (19), termed H-DNA given its homo purine (hPu)/homo pyrimidine (hPy) nature, also refer- ring to hinged DNA) (20), first identified in RNA in 1957(21) before being characterized in DNA in 1979; (22) the G-quartet in 1962 (23), the constitutive unit of the quadruple helix G-quadruple (G4-DNA, or G-DNA) whose formation was demonstrated in 1988; (24) the tetra-stranded four-way DNA junction proposed as a model to explain

gene conversion in 1964 by Robin Holliday (consequently called the Holliday junction) (25), predicted in 1966 (26) and demonstrated in vitro (and termed cruciform DNA, C- DNA) in the early 80s; (27,28) the Z-DNA first detected in 1967 (as a B-DNA of inverted, left-handed helicity) (29) be- fore being firmly confirmed in 1979 (30), etc.

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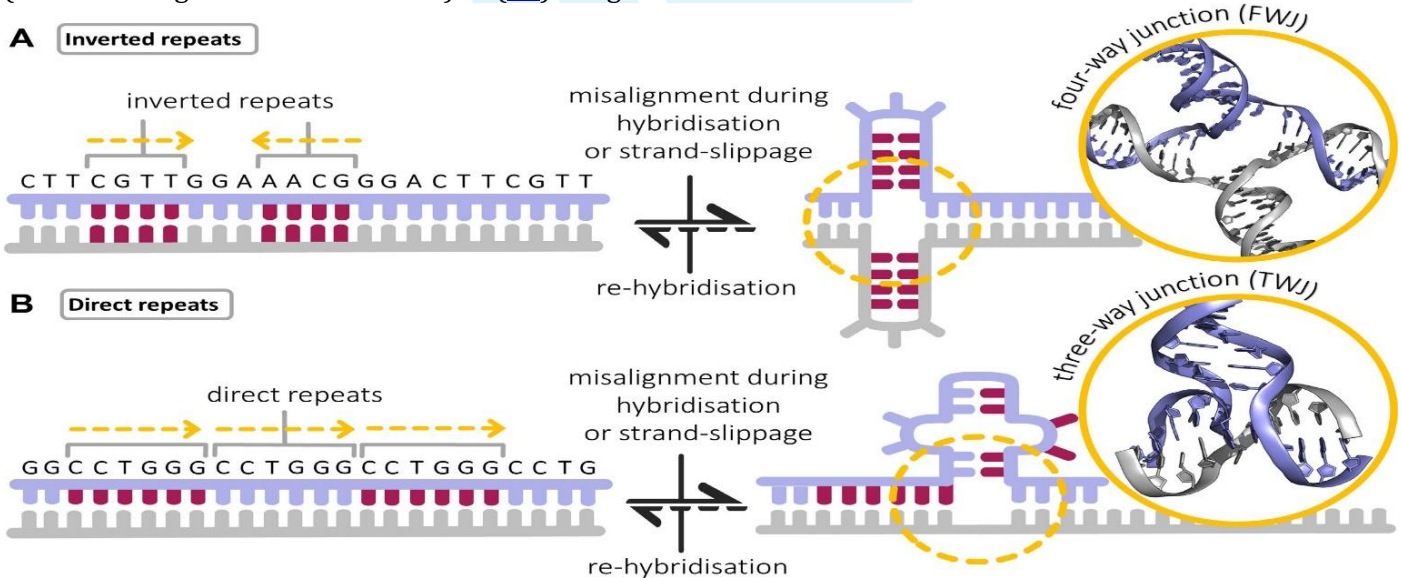
The reason why these alternative structures have been overlooked for years is that their existence in a cellular con- text is challenged by the chromatin environment, in which the dense DNA packaging massively favors B-DNA. This has made the demonstration of their existence in vivo- like conditions daunting, and the chemical biology tools required to do so have reached the necessary degree of maturity only recently (e.g. the visualization of G4s in fixed cells by the antibody BG4 in 2013 (35), in live cells by the small molecule SiR-PyPDS in 2020) (36). Also, the rules that dictate their formation in the genome are poorly under- stood even if the sequences these structures fold from (i.e. repeated sequences) are known to be both abundant and widespread in the human genome (repetitive DNA elements cover roughly half of our genome) (37,38). Non-B-DNA-prone sequences can be inverted repeats (IR, involved in the formation of four-way DNA junction (FWJ), or cruciform DNA, Figures 1 and 2A), direct repeats (DR, involved in the formation of three-way DNA junction (TWJ), also termed slipped-strand DNA, slipped DNA or S-DNA (39), Figures 1 and 2B), mirror repeats (MR, involved in the formation of H-DNA) (38,40,41), or short tandem repeats (STR, involved in the formation of hairpin DNA, G4-DNA and i- motif (iM or I-DNA, Figure 1) depending of the repeated motif) (42). Thanks to recent computational analyses of an entirely sequenced human genome, the abundance of the non-B-DNA-prone sequences can be calculated: it is established that the least abundant motif is MR, with ca. 70 000 occurrences (ca. 2/100 kb), while the most abundant motif, IR, has >6000000 occurrences (ca. 206/100 kb) (41). How- ever, despite the abundance of sequences that could give rise to non-B-DNA structures, the timing and kinetics of their folding are still poorly understood. Also, this folding requires the involved sequences is to be freed from the duplex structures as shown in the pictorial form. Constraint (i.e. open chromatin, damaged DNA), their formation is thus transient only and subjected to a permanent and careful surveillance by ad hoc enzymes (e.g. helices) (43), which make their formation even less likely. This thus explains why non-B-DNA structures have not been considered as reliable genetic elements (and targets) for years. De- spite all these constraints, some of these structures (G4s, iMs) have been isolated and identified by sequencing methods. Efforts invested in vitro (that is, with purified DNA fragments) confirmed their widespread formation in the hu- man genome (with >500 000 G4- (44) and iM-forming (45). Sequences), but not only (46) (e.g. >25 000 G4- (47) antiM-forming (48) sequences in plants) (49). Investigations performed in vivo distinctly highlighted the suppressive role of chromatin for non-B-DNA structure formation as only ca. 1% of the G4s detected in vitro (G4-seq) (44) were detected in vivo (G4 ChIP-seq) (50). More globally, a combination of foot printing experiments (KMnO4/S1 nuclease) and genome-wide sequencing (ssDNA-seq) allowed to links DNA regions in mammalian cells with non-B-DNA structures (C-, G-, H- and Z-DNA, ca. 20 000 motifs each) and then, these structures with regions involved in gene expression regulation (51). Further combining nuclease cleavage (S1 and P1) and sequencing (S1-END-seq and P1-END- seq, respectively) revealed that C- and H-DNA formation (at dinucleotide (TA)n repeats and hPu/hPy repeats (e.g. (GAA)n), respectively) is both widespread in human cells but also strongly dependent on the cell status (cancerous versus non-cancerous cells) (52,53). These results, beyond confirming the existence on non-B-DNA structures in vivo, provide also a strong correlation between structure-prone sequences and both mutability and genetic instability (52) (often referred to as to RIM, for repeat-induced mutagenesis) (54,55). Indeed, their widespread and no uniform distribution across our genome is significantly enriched in regulatory regions, and the distribution quite often overlaps with that of reported hotspots, i.e. regions prone to undergo breakage, deletions and translocations (38,41,55,56). This explains why repeated sequences are carefully patrolled and tightly controlled by genome surveillance systems, notably the DNA damage response (DDR) machinery (57–59).As indicated above, to adopt higher-order structures, these sequences must be relieved from their duplex constraint and re annealed with misalignment or slippage. Their folding is thus dependent on, and

coupled with, DNA trans- actions (transcription, replication) and repair. This makes them interesting targets for therapeutic approaches aimed at inflicting damage to highly active, that is, rapidly dividing cells. Indeed, once folded, these thermodynamically stable structures might act as roadblocks to polymerases, pausing and/or stalling their processivity, which is recognized as a situation of crisis (DNA damage) (60–63). It is therefore unsurprising that chemical biologists soon envisioned an original strategy in which the transient stabilization of non- B-DNA structures by small molecules (so called ligands) could be exploited to foster this situation of crisis notably in rapidly dividing, that is, cancer cells (62,64). The relevance of this approach is further substantiated by the fact that cancer cells are generally DDR-impaired (57), which makes them more sensitive to DNA damage-inducing agents than healthy cells. This explains why many of the therapeutics currently used in the clinic damage DNA, although through different modalities (65). However, these therapeutics tar- get B-DNA (or their associated proteins such as topoisomerases) but none of them target non-B-DNA structures. This is clearly the major caveat of this field, which de- serves to be addressed soon in order to lend credence to its strategic relevance. The most advanced molecule, the G4-stabilizer CX-5461 is currently in phase I (clinicaltrials.gov NCT02719977) (66) against advanced solid tumors (its parent molecule CX-3543, also known as Quarfloxin, was stopped after phase I). Without a successful example of non-B-DNA targeting agent in the clinic, this field will keep on suffering from a lack of legitimacy. Yet, in contrary to B-DNA, which offers poorly defined binding sites only (i.e. minor and major grooves, intercalation in between 2 bp), non-B-DNA structures offer a broad variety of structurally well-defined ligand binding sites, which makes highly selective targeting with small molecules possible. Chief among them are the G4-DNA (67–69), which display two G-quartets surrounded by flexible loops, and DNA junctions (70–72), in which the junction point between the duplex arms (3 duplex arms for TWJ; four arms for FWJ) creates a cavity prone to welcome small molecules (vide infra). The recent developments described in this review will be focused on the targeting of DNA junctions, as that of G4-DNA is regularly covered by authoritative reviews (68,69,73–77) to which interested readers are invited to refer. These developments offer this field a shining message of hope, as these new ligands allow for a specific targeting of DNA junctions (78), yet in in vitro and cell-based assays only to date, which bears significant potential for delivering soon a new generation non-B-DNA targeting therapeutics for which the demonstration of clinical efficacy is greatly expected.



(Figure-13,FWJ,TWJ)

The canonical, so called Watson–Crick, structure is referred to as B-DNA (as the X-ray crystallographic structure was obtained by Rosalind Franklin after hydration of a first sample ('A') of high-quality DNA provided by Rudolf categorized as a non-B-DNA structure, spanning from A-DNA (thus, dehydrated duplex) (11) to Z-DNA (duplex of inverted helicity, Z for zigzag) (10,15).

The central dogma of biology, heralded by Francis Crick in 1957 (16,17), has placed the B-DNA at the very Centre of all molecular biology efforts invested, and discoveries made, after the elucidation of its double helix structure (2,13,18). This has kept the limelight away from reports published in succeeding years on the ability of DNA to fold into a variety of non-B-DNA structures (Figure 1), including: the triple helix (or triplex (19), termed H-DNA given its homo purine (hPu)/homo pyrimidine (hPy) nature, also refer- ring to hinged DNA) (20), first identified in RNA in 1957 (21) before being characterized in DNA in 1979; (22) the G-quartet in 1962 (23), the constitutive unit of the quadruple- helix G-quadruple

(G4-DNA, or G-DNA) whose formation was demonstrated in 1988; (24) the tetra-stranded four-way DNA junction proposed as a model to explain gene conversion in 1964 by Robin Holliday (consequently called the Holliday junction) (25), predicted in 1966 (26) and demonstrated in vitro (and termed cruciform DNA, C- DNA) in the early 80s; (27,28) the Z-DNA first detected in 1967 (as a B-DNA of inverted, left-handed helicity) (29) be- fore being firmly confirmed in 1979 (30), etc.

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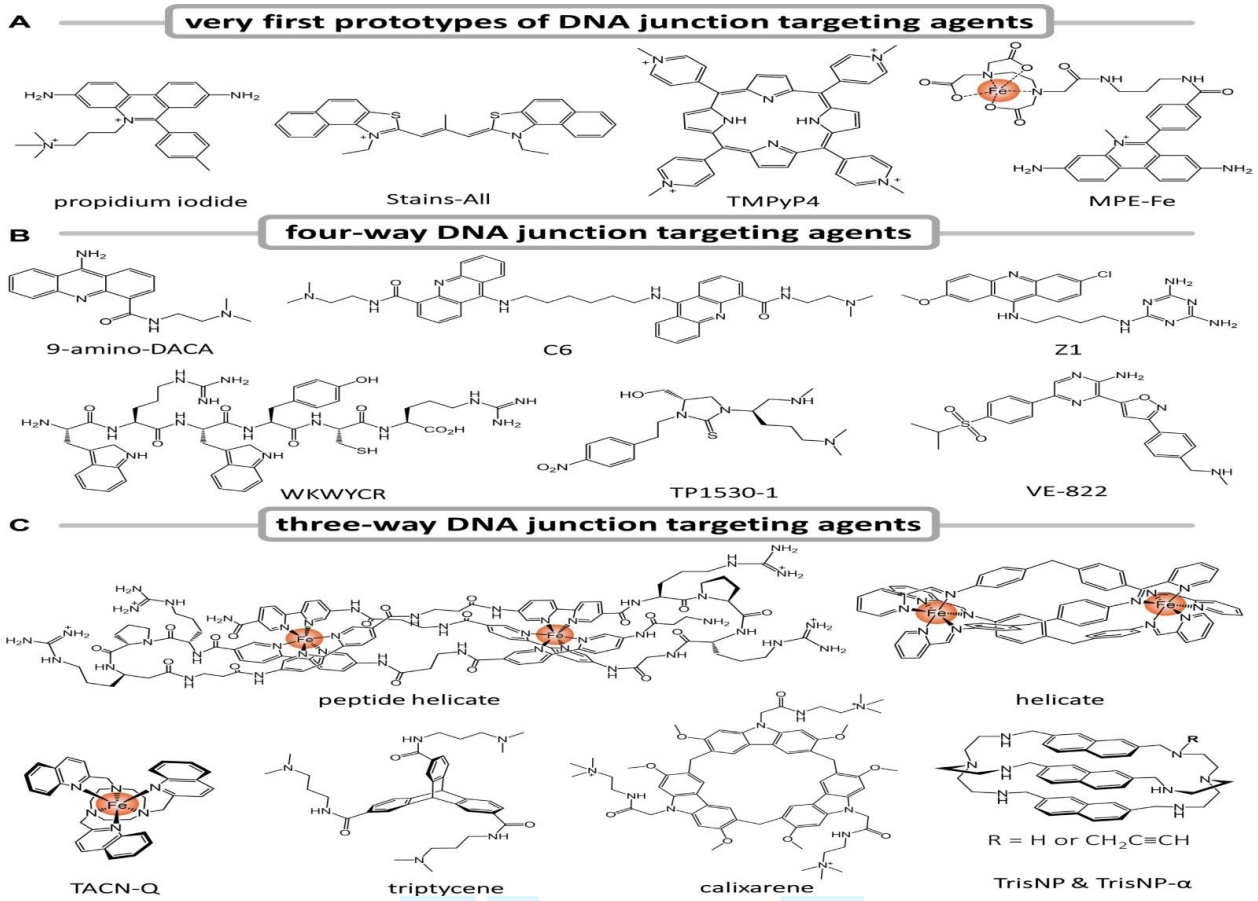
Structure, Functions and Targeting of DNA Junctions

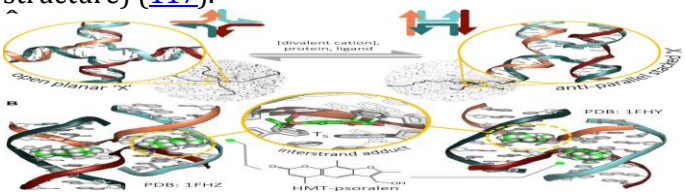
The field of non-B-DNA structures and related ligands was undoubtedly pioneered by Neville Kallen bach (72,79). In a series of articles published in the mid 80's-mid 90's, he pro- vided both biophysical characterizations (e.g. by calorimetric (80), gel electrophoresis (81), chemical foot printing(82,83), etc.) of DNA junctions (but also of G4-DNA) (84,85) and the first insights into how small molecules (Figure 3A), mostly fluorescent dyes, i.e. the DNA labeling agent propodeum iodide (86),the cyanine stains-all (87), the porphyrin TMPyP4 (88), but also cleaving agents such as methidium-propyl-EDTA-Fe(II) (MPE-Fe) (89), interact with them (and with G4s) (90). Of course, these studies were limited to in vitro investigations and the selectivity for DNA junctions over B-DNA, of utmost importance for chemical biology and medicinal chemistry, was not investigated. Anyway, these studies were instrumental in that they spurred on research aiming at ultimately investigating the cellular effects on these new genetic targets and molecular tools (further discussed below).

5.1 Targeting four-way DNA junctions (FWJ)

As indicated above, the existence of a four-stranded DNA structure was postulated by Robin Holliday in 1964 as an intermediate in homologous recombination (HR) (25,91). The possible formation of a central four-way junction intermediate (a 'chiasma') had already been discussed by Joseph G. Gall in 1954 (92), as a basis for the crossing-over mechanism, i.e. the reciprocal exchange of segments along pairs of homologous chromosomes. However, heroic efforts were needed to obtain a structural confirmation of the FWJ organization of this intermediate: various biochemical and physicochemical methods were implemented, including gel electrophoresis (93–95), fluorescence energy transfer (FET (96,97), then reported as FRET (98,99), for fluorescence resonance energy transfer), birefringence decay (100), and nuclear magnetic resonance (NMR) (101,102), along with some imaging techniques such as scanning electron microscopy (SEM) (103) and atomic force measurements (AFM) (104). Among these techniques, X-ray crystallography played a central role: (105) solid state analyses were indeed instrumental to solve the structure of FWJ, alone (106,107) or in interaction with enzymes in charge of the recombination (the re solvase RuvA (108,109) or the re combin as Cre (110), vide infra) (111) and then, small molecules (vide infra). All these techniques concurred in demonstrating the organisation of the FWJ, with its four arms that either extend from opposite sides of the central cavity, which is therefore wide open (the so-called open planar X-structure), or stack on each other two by two (the so- called stacked X-structure), which consequently closes the central cavity (Figure 4A) (70).The initial junction structure was obtained while studying DNA mismatches, and a later study revealed that, in the crystal structure, junction formation is sequence dependent (112). These authors carried out a systematic study of the decamer sequences CCn1n2n3N1N2N3GG, where N can be any of the four common nucleotides, and n are specified accordingly to maintain the IR motif and thus self complimentarily of the sequences. From the 64 possible combinations, they concluded that the d(CCGGTACCGG) sequence gave the junction form under all conditions tested, whereas other sequences could be in equilibrium with A- or B-DNA forms. A way to control the conformation is the covalent binding of psoralen: (113) the closely related sequence d(CCGCTAGCGG) was found to crystallise as a B-form duplex, but the binding of psoralen after laser irradiation at the TA/TA step led to the stabilisation of the junction (Figure 14). More generally, the X-ray structures obtained in presence of enzymes and ligands showed that these effectors can modulate the shape of the FWJ in order to structurally optimise the ligand /DNA association (induced-fit).Regarding the way small molecules interact with finally, the X-ray structure of C6 in interaction with a full FWJ resulting from the self-assembly of the d(CCGGTACCGG) sequence was successfully solved and refined (Figure 5B) (118). This clearly defined binding mode affirmed the suitability of the central cavity of FWJs to accommodate close contacts with dedicated ligands.(FIGURE,14,DNA Junction).

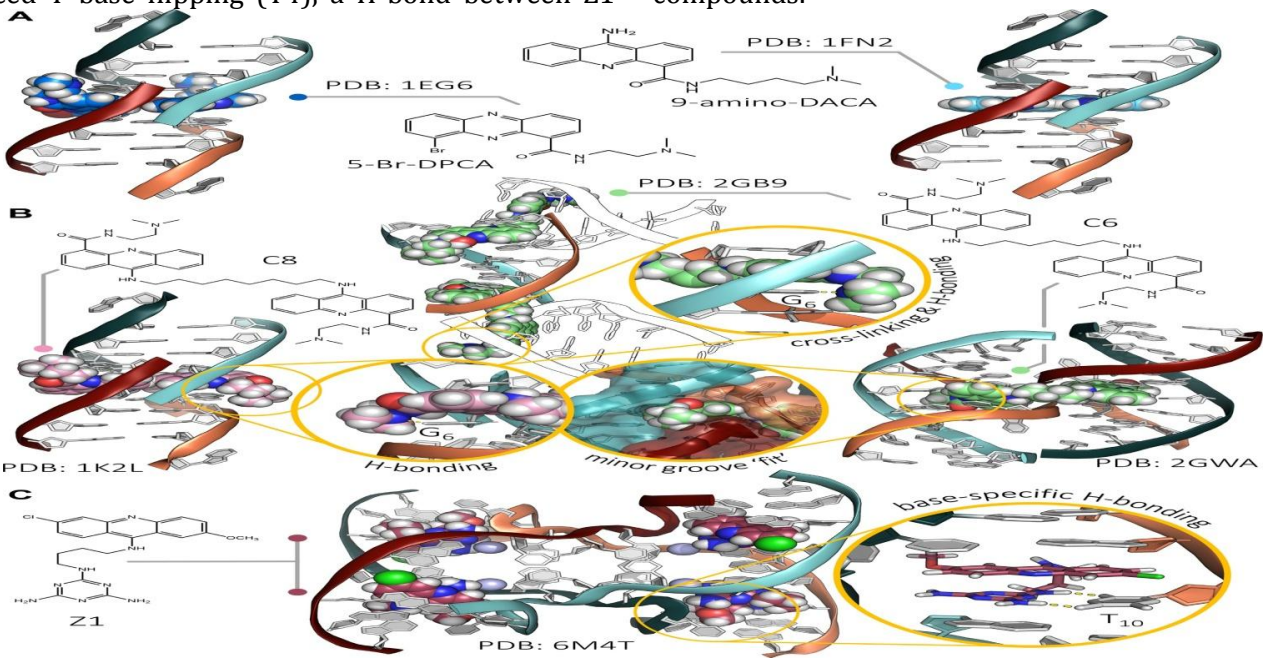


FWJs (Figure 3B), the first insights were obtained through the ligand-induced dimerization of a short, 6-nucleotide (6- nt) duplex. In this study (114), incubation of the self- complementary d(CGTACG) sequence, or the closely related d(CG[5-BrU]ACG), with acridness (e.g. DACA, 9- amino-DACA, Figures 3B and 5A) (114) and phenazine.(115) derivatives provided an asymmetric unit in which the terminal C of one strand invades the second helix at the lig- and binding site, therefore creating a cavity in which two acridness nestle in between four base pairs (Figure 5A). We thus designed dimeric derivatives of DACA (linked through 1,6-diaminohexane or 1,8-diaminooctane linkers, named C6 and C8, respectively, Figures 3B and 5B) as possible bis inter collators and determined two types of X-ray structures with d(CGTACG): a structure in which, behaving similarly to its monomer counterpart (see above), C8 induces a FWJ-like assembly on the basis of a terminal C exchange and creates a H-bond with the residue G6 (Figure 22); (116) and a structure in which C6 cross links two separate duplexes, each of its acridine units being intercalated in between two CG base pairs of a given duplex (Figure-15-X DNA structure) (117).



In this example, the acridine units cause two A residues (A6) at the central TA step of the crossover strands to flip out to gener- ate the ligand binding site in which both a long H-bond (between the DACA sidechain and the residue C7) and stacking interactions (in between a T and a C) with surrounding nucleo bases are created. Later, it was demonstrated that DACA derivatives were also able to induce the formation of FWJs (119); however, these studies, though elegant and insightful, were limited to structural investigations only, owing to the known tendency of acridine derivatives to intercalate within duplexes (120). Their use in cell-based assays would have been troublesome because of their strong B-DNA interaction, which would have blurred the relevance of FWJs as therapeutic target: indeed, it would have been impossible to discriminate the origins of the cellular effects observed between those originating in B-DNA intercalation (the most likely event) and those originating in FWJ inter- action (the less likely event). This is mostly due to the fact that these ligands do not target the unique structural competent of FWJs (i.e. the central cavity), but instead bind to the duplex arms at the proximity of the cavity, which makes specific interaction unattainable. One possibility would have been to structurally fine-tune acridines to preclude intercalation in a manner reminiscent of what was done with G4 ligands, which led to the design of the 3,6,9-trisubstituted acridine BRACO-19 (121). Another possibility was to tar- get

the central cavity of FWJs; as discussed further below, this was done with a series of short peptides but without firm structural characterization of their FWJ binding mode. As with the original Holliday junction structure, which was a serendipitous discovery while studying DNA mismatches, a recent mismatch study also uncovered junction formation (122). A potent DNA-binding compound tri amino otriazine acridine conjugate (Z1, Figure 3B) functions by targeting T:T mismatches in CTG tri nucleotide repeats that are responsible for neurological diseases such as myotonic dystrophy type 1, but its binding mechanism remains unclear. The crystal structure of this ligand was solved in a complex with DNA containing three consecutive CTG repeats with three T:T mismatches. Surprisingly, direct intercalation of two Z1 molecules at both ends of the CTG repeats induced T base flipping (T4), a H-bond between Z1 and a T (T10) and DNA backbone deformation to form a four-way junction (Figure 5C). The core of the complex unexpectedly adopts a U-shaped head-to-head topology to form a crossover of each chain at the junction site. The crossover junction is held together by two stacked G:C pairs at the central core that rotate with respect to each other in an X-shape to form two non planar minor-groove-aligned G:C:G:C tetrads. Two stacked G:C pairs on both sides of the central core are involved in the formation of pseudo-continuous duplex DNA. However, and again, these studies were limited to structural investigations only owing to the lack of information concerning the specificity of Z1 for FWJs that, combined with the known health-threatening nature of triazines (notably widely used as herbicides) (123), is not the best guarantee of a bright future for this series of compounds.



(FIG-16 PDB,H-BONDING)

5.2 The relevance of FWJ in cells

Although the central position of HJs in HR has been firmly established for decades (91), it is only recently that they were visualized in cells (124). While HR is only one of two major pathways leading to double strand DNA break (DSB) re- pair in mammalian cells, along with non-homologous end joining (NHEJ) (Figure 6) (125), it is indispensable in lower organisms (bacteria, yeasts) for ensuring DNA transfer and adapting to evolution (natural selection) (126). It is there- fore unsurprising that a vast body of research has been dedicated to understanding the roles of HJ/FWJ in yeasts and bacteria. These efforts have led to the discovery of the RuvABC complex that operates at HJs in prokaryotes (Escherichia coli) in a sequential manner: RuvA binds to HJs and targets RuvB to the junction, both RuvA and RuvB (RuvAB) promote branch migration and RuvC resolves the junction (127). An engineered synthetic protein RuvCD efGFP was recently used to investigate HJs in cells der [Fe2L3]4+) was misleadingly ascribed to RFs (101,137), while the fine structural details of RFs indicate that it is in- deed a Y-shaped structure but made of unpaired sequences at the junction, unlike TWJs (170). Second, these first cellular investigations failed in providing convincing results, and the ability of [Fe2L3]4+ to interact with DNA in cells was even questioned (189). Third, the very nature of [Fe2L3]4+ made these investigations irrelevant owing to the ability of this supramolecular complex to interact with other DNA structures including the major grooves of B-DNA (191) and G4s (122,143). Therefore, this prototype was invaluable to uncover and accurately characterize a completely new DNA binding mode (with a molecule nestling within the central cavity of a TWJ) but was not ideally suited for chemical biology investigations. A very interesting

observation, however, was that the closely related cylinder [Ru2L3]4+ was able to inhibit DNA transactions by preventing polymerase processivity; (194) this laid the basis for investigating whether TWJs might fold during DNA transactions in cells, more particularly at replication sites in the nucleus. To this end, co-localization studies were performed with a fluorescently labelled Fe(II) helicity (TAMRA-LLD, Figure 8B) and PCNA (proliferating cell nuclear antigen, a component of the replication machinery) fused to the green fluorescent protein (GFP) in

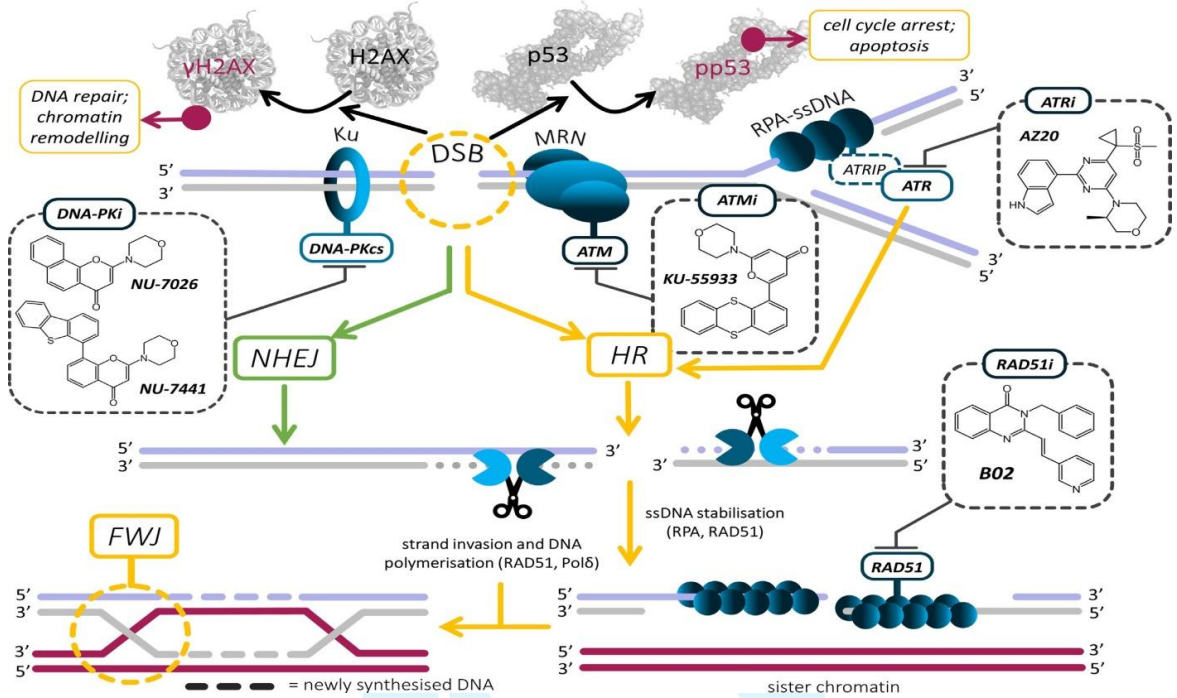


FIGURE-17, Synthesis of new DNA.+

HeLa cells (177). The collected images showed well-defined common foci, indicating that TWJs might fold transiently during DNA replication, in the vicinity of the replisome, and be trapped by ad hoc ligands. Similar co-localization studies were recently performed with a TWJ probe comprising a central, C3-symmetrical florigenic template (1,3,5-tristyrylbenzene) surrounded by three peptide arms designed to interact with the three duplex arms of the TWJs (AT-hooks), thus found to preferentially accumulate in permeable cells where PCNA-GFP accumulates (195). However, the relevance of these results needs to be substantiated by a clear demonstration of the selectivity of used ligands for TWJs, as off-target effects cannot be ruled out on the basis of the currently available in vitro data. Studies were also performed to investigate whether TWJ ligands trigger DNA damage in dividing cells. This was firmly established via the immune detection of γ H2AX by both optical imaging and flow cytometry: after a thorough characterization of their TWJ-interacting properties in vitro (affinity and specificity via a panel of different techniques), two aza cryptands (TrisPOB, TrisNP) were studied in cells and shown to trigger an accumulation of DNA strand breaks (and particularly DSBs) (Figures 3C, 8C and 9) (155,156). When combined with the established ability of TWJ ligands to prevent polymerase activity in vitro (194), these results thus provided a strong rationale for a mechanistic model in which ligand-stabilized TWJs act as road- blocks that hamper proper processivity of DNA-related enzymes, slowing or even stopping their motion along the genomic duplex, which is recognized as a DNA damage and trigger the DDR and repair machineries. We further exploited this model by using drug combinations in which TWJ ligands were used to induce DNA damage and DNA repair inhibitors to act synergistically with them, in an approach referred to as chemically induce synthetic lethality (176). In eukaryotic cells, DSBs can be repaired by either HR (vide supra) or non-homologous end joining (NHEJ). (Figure 6). The former relies on a broad array of protein effectors including RAD51 (which binds to single-stranded DNA, searches for sequence homology and favours strand exchange), and the kinases ATM and ATR (which bind to DSBs and stalled RFs, respectively); the latter also involves multiple processing enzymes, including kinases and ligases (e.g. DNA-PK and Lig4, respectively) (58,59,125). The ant proliferative activity of the TWJ ligands TrisPOB and TrisNP was found to be quite efficiently potentiated when combined with inhibitors of DNA-PK (NU-7441), ATM (KU-55933) and RAD51 (B02) (155,156), confirming that the cellular activity of TWJ ligands relies n the induction of strongly geno toxic DSBs (Figure 9). Interestingly, a synergy was also obtained with the Top2 inhibitor BNS- 22: Top2 proteins resolve DNA topological stress (197) but are also involved in the recognition of alternative DNA structures and the formation of DSBs at these sites (198). Top2 also participates in the cleavage of hairpin structures formed from α-satellite sequences

in vitro (199), which are Centro metric regions known to be highly repetitive (200) and to fold into secondary structures (201). This synergy, con- firmed by cyto toxicity assessments and γ H2AX labelling, thus implies that Top2 inhibition favours TWJ formation, which was further demonstrated unambiguous interpretation of cellular outcomes. To con- firm this interpretation, we showed by

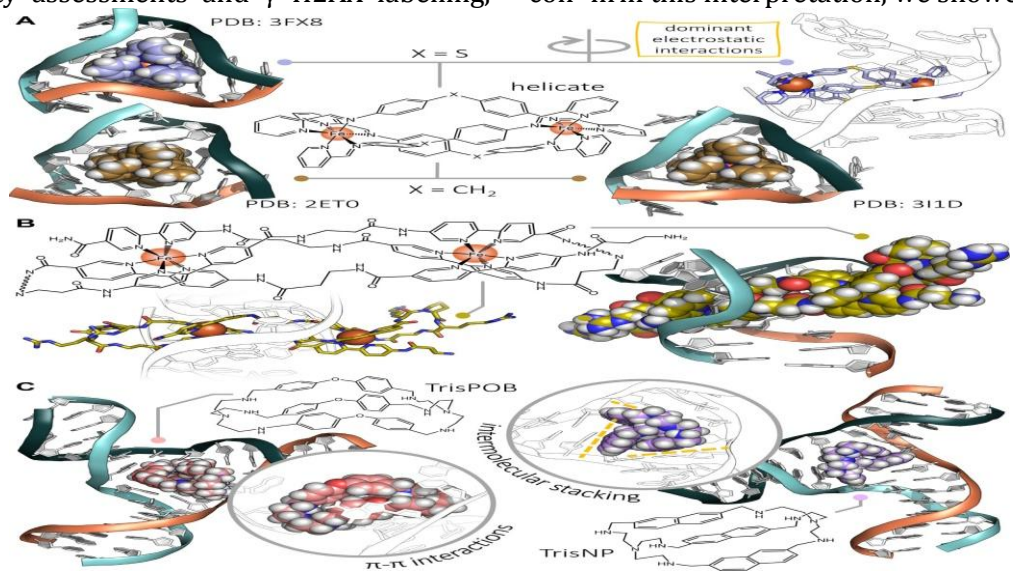
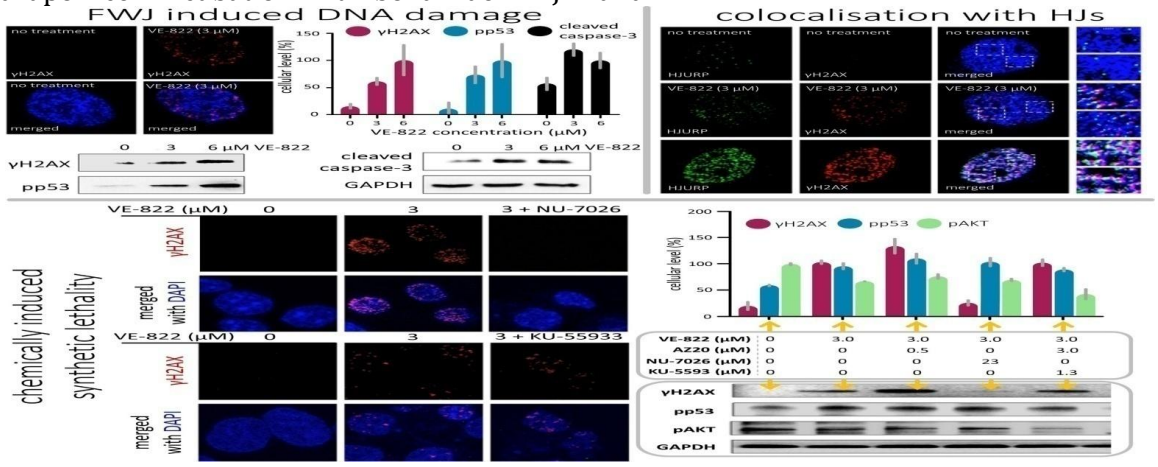


Figure-18,PDB,TWJ

by bio orthogonal chemistry using the in situ clickable TWJ ligand TrisNP-α, Figure 3C) (156), which highlight the increase of the TWJ landscape (TrisNP-α labelling) upon Top2 inhibition (Figure 9). This opens brand new therapeutic opportunities, notably to treat cancers resistant to Top2 poisons. As above, though comprehensive from a molecular and cellular biology point of view, these studies suffer from the fact that used TWJ ligands display un neglect able affinity for G4s. It was demonstrated in vitro that they display a preferential affinity for TWJ (i.e. in competitive setups where both TWJ and G4 are mixed, the ligands interact solely with TWJ) but this selectivity was not satisfying enough for an optical imaging that there is no overlaps between TWJ sites labeled with TrisNP-α (in situ click chemistry (182) with AF594-azide) and G4 sites labeled with the G4-specific antibody BG4 (35).These results are highly convincing but they are still not strong enough to unequivocally dispel doubts about the actual ori- gins of the cellular effects monitored upon cell incubation with bona fide TWJ ligands. What the field now needs is a truly specific TWJ ligand; in light of the wealth of promising data described above, we can wager that a substantial research effort is currently being invested to identify the impatiently awaited game-changing TWJ ligand (128). RuvCDefGFP is a catalytically defective (Def) RuvC fused to green fluorescent protein (GFP); it was used to both map HJs in E. coli by HJ-ChIP-seq (using the anti- RuvC antibody), which demonstrated the accumulation of HJs near DSB sites, and visualize HJs via live-cell imaging, with a distribution correlated with homology-directed DSB repair, notably at single-stranded gaps.

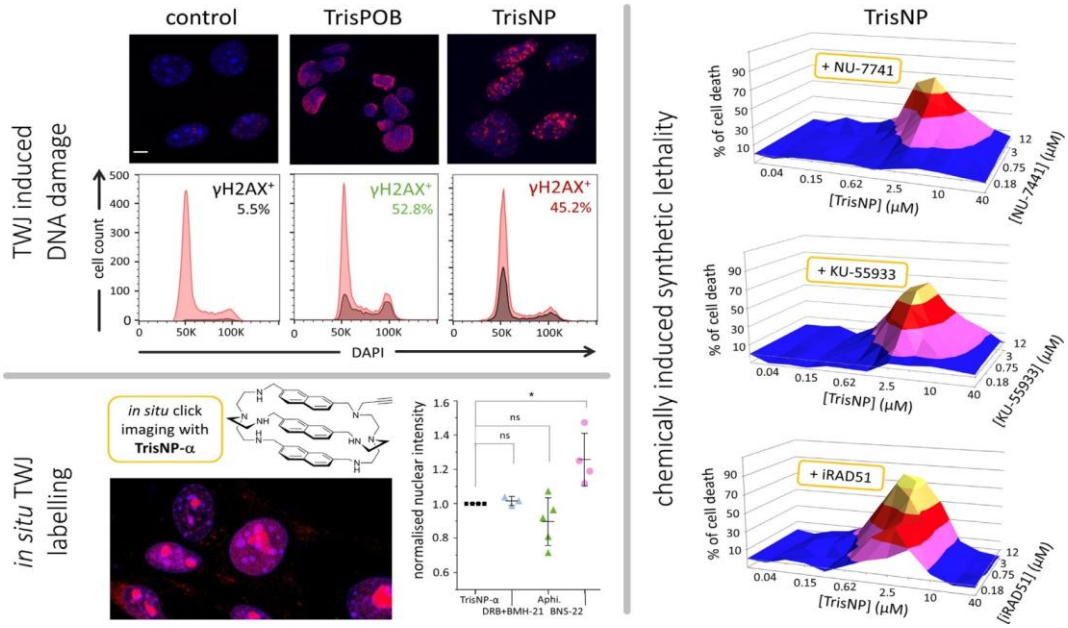
These compelling results thus lend further credence to value of targeting HJs in bacteria bysmall molecules. The short hex peptides developed by Anca M. Segall and co-workers were among the first reported HJ-ligands. It was first demonstrated that WKHYNY (Trp-Lys-His-Tyr-Asn- Tyr) target HJs in vitro and inhibits its resolution by the re-combination proteins integration host factor (IHF, a hetero-dimeric protein that binds to and bends DNA and RNA.



(Figure-19 FWJ OF DNA)

6. Conclusion and Future Directions

Targeting higher-order DNA structures with ad hoc ligands for chemical biology and/or medicinal chemistry purposes is now a commonly accepted strategy, undoubtedly as a consequence of the wealth of data accumulated aboutG4-DNA (68,69), the first-in-class example of a biologically relevant alternative DNA structure. While DNA junctions were discovered concomitantly with G4s (as indicated above, their basic structural unit was solved in 1962 (23) but their biological relevance was only discussed in the late 1980s) (24,178,181), the investigations aiming at confirming the existence of DNA junctions in human cells, scrutinizing their functional relevance and establishing the reliability of the therapeutic strategies based on their targeting with small molecules still . This might: first and fore-most, right after the pioneering report by Stephen Neidle, Lawrence H. Hurley and co-workers on a small molecule able to interact with a G4 (in the aim of indirectly inhibiting telomerase via the sequestration of its telomere substrate under a form that is not recognized by the enzyme) (175), hundreds of G4 ligands have been synthesized and studied (75,168). This incredible enthusiasm has provided as strong pet us for the discovery of ligands (e.g. PhenDC3 (151), PDS) (140) which have some biology in a (159). The DNA junction field has not experienced such a keen interest, presumably because the biological relevance of DNA junctions is still poorly understood. Also, the search for genomicG4 sites via in silicon techniques (e.g. Quad Parser (180),G 4hunter (171), etc.) was straight forward thanks to the very nature fG4-forming sequences (example of used algorithm: $d(G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+})$)(162).They have provide strong arguments about the prevalence of G4s in the human genome, which was soon connected to widespread functional relevance. The nature of the DNA junction-forming sequences (direct or inverted sequence repeats, and not easily identifiable moiety repeats such as GGGtriplets) makes them more difficult to be reliably predicted at a genome-wide scale (although some laudable attempts have been made, such as IR finder (153), palindrome analyzer (144), etc.). Again, this has contributed to some-what dampen enthusiasm for the search of genomic DNA junction-forming sites. Finally the challenge of identifying genomic G4 sites by ChIP seq like techniques (such as G4ChIP-seq) (50) was met, thanks to the development of the G4-specificantibodyBG4(35),and no similar antibody exists in the DNA junction field, which makes both their identification (ChIP) and visualization (immune fluorescence) challenging and regularly questioned. Despite the difficulties, the aforementioned efforts have started to pay off, but the prevalence of putative DNA junction forming sequences described above (37,41) combined with the lack of fine details regarding DNA junction biology (which originates in the lack of reliable molecular tools) explain why this chemical biology quest is still in its infancy. Massive efforts must now be invested to keep on developing tools and technologies to interrogate and manipulate DNA junctions in a functional cellular context. The next steps will thus be to (i) identify truly specific ligands for DNA junctions, in order to establish are liable link between junction interaction in cells and the cellular outcomes monitored; (ii) create and/or optimize in vitro screening as says to blind screen commercially available chemo libraries in order to expand the portfolio of DNA junction ligand candidates far beyond rational design; (iii) develop on the basis of identified promising scaffolds multivalent molecular tools (bearing for instance an alkynes appendage that could be manipulated in a bio orthogonal manner in cells) to identify the sequences they interact with (direct repeats? Inverted repeats? Other?) And the protein as associated combining both sequencing (bioinformatics tools will have to be optimized as well) and proteomics. Of note, the identification of DNA junction-binding proteins will be a very important step as they are the cellular effect or by which the response to junction stabilization by adhoc ligands is mediated; while hundreds of G4-binding proteins are now known(175),only a handful of junction-binding proteins have been reported to date (e.g. RuvABC and RecG, *vide supra*); (iv) produce DNA junction-specific antibodies for both immune precipitation and immune detect ion purposes, and(v) develop cellular and small animal models to assess the properties of identified candidates in a standardized manner. The ultimate goal of the seen investigations will be to rapidly provide a proof-of-concept that DNA junction-target in molecules can be considered as key players in the field of the rape tic agents, to validate all the necessary pre-clinical milestones in a reliable manner in the aim of reaching the clinical stage rapidly and confidently. Without a doubt, the chemical biology investigations will to major advances in research on this new class of therapeutic targets, a momentum that will contribute to both better understand the biology of DNA junctions (which will find applications in diverse the rape tic areas such as cancers (62), neuro pathologies (165) and in factious diseases) and unravel the fascinating structural and functional diversity of DNA.



Anew Drug Design strategy in the light of Molecular Hybridization Concept of DNA/RNA small molecules attachments:

Molecular hybridization of nucleic acids is the process in which two single-stranded nucleic acid molecules with complementary base sequences form a double-stranded nucleic acid molecule. Nucleic acid hybridization technology is a fundamental tool in molecular biology, and has been applied in various fields such as detection of gene expression, screening specific clone from DNA or genomic library, determining the location of a gene in chromosome and diagnosis of diseases like property for eg grapheme or glue like bio material from spider or even polymer can bind rapid growth of cell for its binding nature at micro level or can trap bacterial or viral growth by trapping within Net like structure. The idea of Quantum Dots may be implied to identify such net as drug to separate from diseases infected Zone. Sometime electrification or by application of Radioactivity through this net may reduce harmful diseases spreading.

Molecular hybridization a useful tool in the design, trapping of new generation drug prototypes using the sequencing of Nucleic acids for a particular patient. Molecular hybridization is a new concept in drug design and development based on the combination of pharmacophores moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy, when compared to the parent drugs. Additionally, this strategy can result in compounds presenting modified selectivity profile, different and/or dual modes of action and reduced undesired side effects. So, in this paper, we described several examples of different strategies for drug design, discovery and pharma co modulation focused on new innovative hybrid compounds presenting analgesic, anti-inflammatory, platelet anti-aggregating, anti-infectious, anti-cancer, cardio- and neuro active properties.

By this molecular hybridization concept in the field of genetic engineering and as well as molecular biology, formation of a partially or wholly complemen tary nucleic acid duplex as association of single strands, usually between DNA and RNA strands (previously associated or un associated DNA, RNA strands.). These are used to detect an isolated specific genetically initiated sequences as measured sequencing homology, or define other characteristics of one or both strands. If we can detect the particular cause of critical disease like Parkinson, Cancer or AIDS for a particular patient. We can synthesis the new drug design for that patient to treat them for complete cure in this molecular hybridisation concept. So this is a new generation concept for developing drug as well as the cause of the particular diseases for a particular patient. Not only that by fusing more than one drugs structure components by Molecular hybridisation concept we can make a single drug for a patient with multiple affected diseases using computer simulation more modern method in the field of medicinal chemistry. The idea will show us to find the way of sustainable health and immortality in future.

Brief Conclusion from Small molecule (targeting Agents) addition with DNA/RNA

Targeting higher-order DNA structures with *Adhoc ligands* for chemical biology and/or medicinal chemistry purposes are now a commonly accepted strategy in drug design.

Folding of DNA/ RNA structure as per need in genetic modification to develop GMO.

Cloning of DNA/RNA for treatment of various diseases as per treatment of various diseases.

GI. Publication and presentation:

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