Kwaghe International
Journal of Sciences and Technology

ISSN : 0000-0000

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/KIJST.v1i1.3400

Review on DNA Modification for Drug Tartgeting Purposes

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jun 19, 2024	Jul 13, 2024	Jul 16, 2024	Jul 19, 2024

Abstract

DNA (deoxyribonucleic acid) modification for drug targeting purposes is a rapidly advancing field that holds great promise for revolutionizing the way we approach disease treatment, offering precise and personalized approaches to tackle diseases. This seminar work provides an overview of the potential application of DNA modification for drug targeting. The seminar work begins by introducing nucleic acid and its types, structure of DNA and DNA modification, the concept of DNA modification and its significance in the context of drug targeting. It highlights the potential of DNA modification techniques, such as gene editing and epigenetic modifications. It also explores the application of DNA modification for drug targeting purposes. It discusses the use of gene editing technologies, such as CRISPR-Cas9, in correcting genetic mutations associated with inherited disorders or targeting disease-causing genes. By utilizing DNA modification techniques, drug targeting can be fine-tuned to enhance efficacy and minimize side effects. In general, this seminar report emphasizes the great potential of DNA modification for drug targeting purposes. By precisely modifying DNA, scientists can develop targeted therapies, correct genetic mutations, and optimize drug responses.

Keywords: DNA, Modification, Drug, Targeting, Purpose

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Introduction

The remarkable structure of deoxyribonucleic acid (DNA), from the nucleotide up to the chromosome, plays an important role in its biological function. The ability of DNA to function as the material through which genetic information is stored and transmitted is a direct result of its elegant structure (Ghannam *et al.,* 2022). In their seminal 1953 paper, Watson and Crick unveiled two aspects of DNA structure: pairing the nucleotide bases in a complementary fashion (e.g., adenine with thymine and cytosine with guanine) and the double-helical nature of DNA (Watson & Crick 1953).

Researchers have argued the discovery of DNA to have been about 145 years ago, with a the more discovery being 70 years ago, all thanks to the work of Avery, MacLeod, McCarty, Watson, and Crick (Dahm 2005). Since then, over a million published works can be found on the subject of DNA, which is the instructions manual of life (Liyanage *et al.,* 2014).

DNA is the molecule that encodes our genes (also known as the genotype) and has been completely sequenced in the "Human Genome Project" as of 2003 (NHGRI, n.d). Genes are however not always expressed, DNA is not always transcribed into ribonucleic acid (RNA), and RNA is not always translated into proteins. This complex network of gene regulation can partly be explained by epigenetics. The term was first coined in 1942 by Conrad Waddington, defining it as "causal interactions between genes and their products which bring the phenotype into being". Epigenetics represents the many inheritable chemical "marks" found on and surrounding the genome that influence gene expression in a controlled and selective manner. DNA methylation and histone post-translational modifications (PTMs) are two epigenetic mechanisms involved in the regulation of gene expression (Hallgrímsson & Hall, 2011). DNA methylation was initially found and extensively studied in prokaryotic DNA since the 1960s. In 1977, 5-methylcytosine (5mC) was identified in eukaryotic DNA by Razin and Cedar (Razin, and Cedar 1977; Pollack *et al.,* 1980; Razin and Riggs 1980). Since their discovery, close to 33,000 published works can be found on DNA methylation, forever changing genetic and chromatin-based research (NIH 2012).

Deoxyribonucleic Acid (DNA)

A nucleic acid refers to any of the group of complex compounds made up of linear chains of monomeric nucleotides. Each nucleotide component, in turn, is made up of phosphate group, sugar, and a nitrogenous base. Nucleic acids are involved in the preservation,

replication, and expression of hereditary information. Two major types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

Deoxyribonucleic Acid (biology definition): A helical double-stranded nucleic acid that is crucial for containing the genetic information for cell growth, division, and function. Abbreviation: DNA. Variant: deoxyribonucleic acid. Synonyms: deoxyribose nucleic acid; deoxyribose nucleic acid.

Structure of DNA

DNA is a polynucleotide; it is made up of several monomeric units of nucleotides covalently bonded by 3', 5' phosphodiester linkages. This means that the 5'-phosphoric group of one nucleotide is esterified with the 3'-hydroxyl of the adjoining nucleotide. Each nucleotide, in turn, consists of phosphate group, a deoxyribose sugar (5-carbon), and a nitrogenous base. The nitrogenous base or nucleobase may be a cytosine (C), guanine (G), adenine (A), or thymine (T). The two strands that make up the DNA form a helical structure. At the core, the nucleobases are complementarily paired. The base-pairing rules are as follows: adenine pairs with thymine whereas cytosine pairs with guanine. The bond that joins the two nucleobases is hydrogen bond. The two strands are antiparallel, which means they run in opposite directions to each other.

DNA molecule has two regions: the coding region and the non-coding region. The non-coding region, as the name implies, is the section of the DNA that does not code for a protein. In a eukaryotic cell, DNA is organized into a chromosome inside the nucleus. The DNA inside the nucleus is compacted by chromatin proteins (e.g. histones). Some of the DNAs are stored in mitochondria (referred to as mitochondrial DNA, mtDNA) and chloroplast (referred to as chloroplast DNA, cpDNA). In a prokaryotic cell, DNAs are found in a special region in the cytoplasm called a nucleoid.

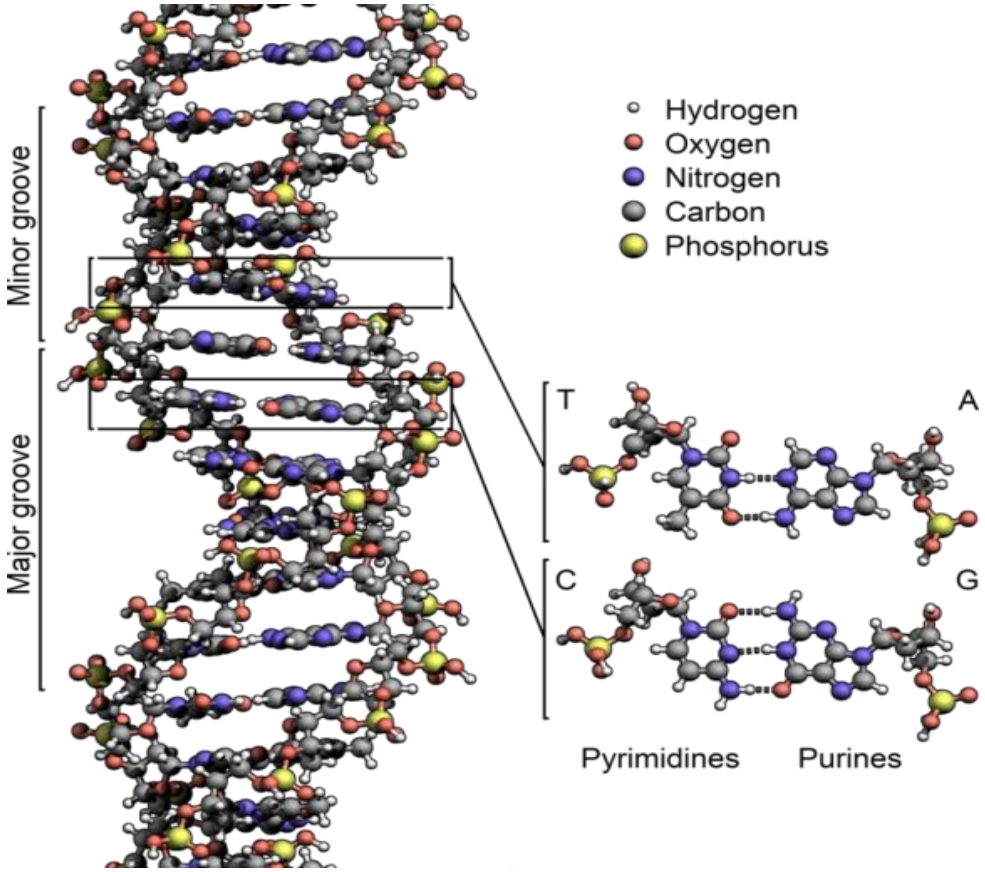


Figure 1: schematic diagram of DNA structure

Source: https://www.biologyonline.com/wp-content/uploads/2019/10/DNA-structure-580x576.png

DNA vs RNA

DNA is a double-stranded nucleic acid containing the genetic information of a living thing. It is essential for the cell growth, division, and function of an organism. RNA is, in general, a single-stranded nucleic acid. Below is a table that summarizes the major differences between DNA and RNA.

Table 1: summary of major difference between DNA and RNA

DNA	RNA
Structure: DNA is composed of two strands that twist together to form a helix, forming a ladder-like structure. Each strand consists of alternating phosphate (PO4) and pentose sugar (2-deoxyribose), and attached to the sugar is a nitrogenous base, which can be adenine, thymine, guanine, or cytosine. In DNA, adenine pairs with thymine and guanine with cytosine. Not all DNAs are double-stranded. For instance, a group of viruses has a single-stranded DNA genome.	**Structure:** RNA consists of a long linear chain of nucleotides. Each nucleotide unit is comprised of sugar, a phosphate group, and a nitrogenous base. It differs from DNA in having ribose as its sugar, (deoxyribose in DNA) and the bases are adenine, guanine, cytosine, and uracil. In RNA, adenine pairs with uracil and guanine with cytosine. RNAs are single-stranded except for certain viruses whose genome consists of double-stranded RNA.
Location: In eukaryotes, most DNAs are located in the nucleoli and chromosomes in the nucleus. A small fraction of the total DNA is present in mitochondria, chloroplasts, and cytoplasm. In prokaryotes and viruses, DNA is found in the cytoplasm.	**Location:** In eukaryotes, RNA is found in the nucleus and in the cytoplasm. In prokaryotes and viruses, it is found in the cytoplasm.
Function: DNA is a long polymer of nucleotides to code for the sequence of amino acids during protein synthesis. DNA carries the genetic 'blueprint' since it contains the instructions or information (called genes) needed to construct cellular components like proteins and RNAs.	**Function:** In some viruses, RNA is the genetic material. For most organisms, RNAs are involved in: *protein synthesis* (e.g. mRNA, tRNA, rRNA, etc.), *post-transcriptional modification,* or *DNA replication* (e.g. snRNA, snoRNA, etc.), and *gene regulation* (e.g. miRNA, siRNA, tasiRNA, etc.).

Source: https://www.biologyonline.com/dictionary/deoxyribonucleic-acid

Prokaryotic and Eukaryotic DNA

Prokaryotic and eukaryotic DNA carry genetic information for the development, functioning and reproduction of prokaryotes and eukaryotes respectively. Eukaryotes consist of membrane bound nucleus whereas prokaryotes lack a membrane bound nucleus. Prokaryotic DNA is double stranded and circular. But eukaryotic DNA is double strand and linear. The amount of DNA in prokaryotic cells is much less than the amount of DNA in eukaryotic cells. Both prokaryotic and eukaryotic (Lakna, 2017).

DNA undergo replication by the enzyme DNA polymerase. The main difference between prokaryotic and eukaryotic DNA is that prokaryotic DNA is found in the cytoplasm whereas eukaryotic DNA is packed into the nucleus of the cell.

Prokaryotic DNA

The DNA which is carried by prokaryotes is called prokaryotic DNA. Prokaryotic DNA is found in the cytoplasm of bacteria. Some prokaryotic DNA is found as the circular plasmids (Nagawa, n.d.), carrying additional information. That means prokaryotic DNA does not contain an enclosing nuclear membrane. Prokaryotic DNA is packed into a single circular chromosome. It resides in the region called nucleoid in the cytoplasm. Nucleoid associated proteins are involved in the packaging of the prokaryotic chromosome in the nucleoid. They help prokaryotic DNA to form a looped structure (Lakna, 2017).

The size of the prokaryotic DNA is around 160,000 to 12.2 million base pairs, depending on the species. Prokaryotic DNA contains a small number of genes. Functionally related genes are organized into operons. Since prokaryotic DNA is rich with genes, the amount of nonfunctional DNA is less. Prokaryotic DNA replication is relatively simple. Prokaryotic chromosome contains a single origin of replication where the initiation of DNA replication occurs. Therefore, a single replication folk and bubble is formed during the replication. The speed of the replication is relatively high in prokaryotes, 2000 nucleotides per second.

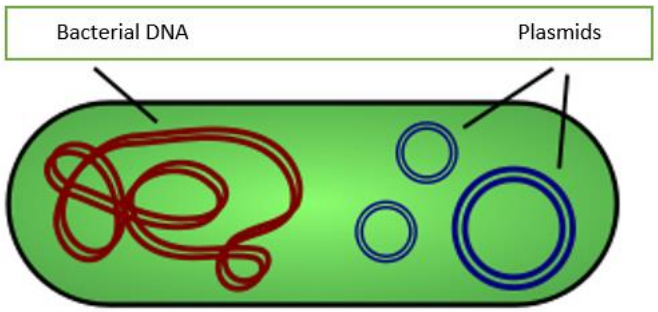


Figure 2: prokaryotic DNA

DNA polymerase is the enzyme involved in the DNA replication; this contains seven different enzyme families. Out of the seven DNA polymerase families, both prokaryotes and eukaryotes share three families of DNA polymerases: DNA polymerase A, B and Y. DNA polymerase C family is only contained by prokaryotes. Pol III is a replicative DNA polymerase, which belongs to the DNA polymerase family C.

Eukaryotic DNA

Eukaryotic DNA is packed into bundles of chromosomes, each consisting of a linear DNA molecule coiled around basic (alkaline) proteins called histones, which wind the DNA into a more compact form. Prokaryotic DNA is found in circular, non-chromosomal form. In addition, prokaryotes have plasmids, which are smaller pieces of circular DNA that can replicate separately from prokaryotic genomic DNA. Because of the linear nature of eukaryotic DNA, repeating non-coding DNA sequences called telomeres are present on either end of the chromosomes as protection from deterioration (Libretexts, 2022).

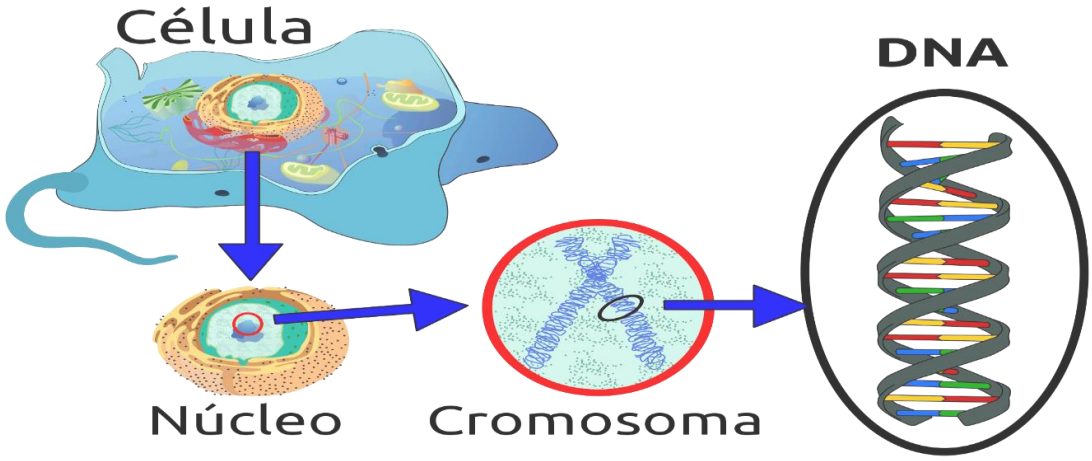


Figure 3: Eukaryote DNA;

Source:https://commons.wikimedia.org/wiki/File:Eukaryote_DNA-es.svg#/media/File:Eukaryote_DNA-en.svg

Table 2: Difference between Prokaryote and Eukaryote DNA

Prokaryotic DNA	Eukaryotic DNA
Location	
Found freely in the central portion of the cytoplasm.	Found within the nucleus.
Occurrence	
Occurs as a covalent closed circular form of DNA.	Occurs as a linear form of DNA with two ends.
Size	
The size of the DNA is less than 0.1 pg in a prokaryote.	The size of the DNA is high, usually more than 1 pg.
Introns	
Introns are absent in the coding region of DNA.	Introns are present in the coding region of DNA.
Nucleosome	
There is no formation of nucleosomes.	There is a formation of nucleosomes.
Quantity of the DNA	
The quantity of DNA is comparatively less.	The quantity of the DNA is more.
DNA Replication	
DNA replication occurs in the cytoplasm of the cell.	DNA replication occurs within the nucleus of the cell.
Number of Genes	
Prokaryotic DNA contains a small number of genes.	Eukaryotic DNA contains a large number of genes.
Transposons	
Prokaryotic DNA lacks transposons.	Eukaryotic DNA consists of transposons.
Number of Chromosomes	
Prokaryotic DNA is organized into a single chromosome.	Eukaryotic DNA is organized into many chromosomes.
Histone Proteins	
Do not interact with the histone proteins.	Associated with the histone proteins.

Literature Review

History and Terminology

Friedrich Miescher 1844 –1895, a Swiss physician and biologist was the first to isolate DNA from the pus of discarded bandages. The then-novel biological molecule was neither a carbohydrate, nor a protein, nor a lipid from the nuclei of white blood cells. He named the compound nuclein from where he was able to isolate it (Biology Online 2022). The acidic properties of the compound were discovered by the German chemist, Albrecht Kossel 1853 –1927. He was also known to be the first to identify the nucleobases: adenine, cytosine, guanine, thymine, and uracil. Later, nuclein was replaced with nucleic acid; the term was coined in 1889 by the German pathologist, Richard Altmann 1852 –1900. (Gribbin, 2002). The nuclein discovered by Miescher was later particularly identified as DNA. The double-helical model of DNA was attributed to the joined effort of molecular biologists James Watson (American) and Francis Crick (British) in 1953. Their double-helical DNA model was based largely on the information that nucleobases were paired and on the X-ray diffraction image (referred to as Photo 51) by Rosalind Franklin 1920 – 1958 and Raymond Gosling in 1952. Francis Crick was also known for laying out the central dogma of molecular biology. His central dogma depicts the relationship between the nucleic acids DNA and RNA, and proteins. With it, Crick showed how the information would be irreversibly transferred from nucleic acids to proteins. Furthermore, he and his colleagues suggested that the genetic code was read according to codons where each codon consisted of three nucleobases. Indian-American biochemist Har Gobind Khorana 1922 – 2011, American biochemist Robert William Holley 1922 –1993, and Jewish American biochemist and geneticist Marshall Warren Nirenberg 1927 –2010 were able to decipher the genetic code and its relevance in protein synthesis. (Nobelprize, 2013). In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty's experiment helped set the DNA as the genetic material, which during their time it was largely believed to be protein.

Cellular Level of DNA

One significant difference between prokaryotes' and eukaryotes' DNA structure is that prokaryotic DNA molecules are circular and thus do not have free 5' and 3' ends. Circular DNA molecules are also found in eukaryotic mitochondrial and chloroplast DNA, evidence that supports the endosymbiotic theory of eukaryotic evolution (Archibald, 2015). In contrast, the ends of eukaryotic DNA molecules do not connect and are thus "free."

Prokaryotes typically have one main circular chromosome, while eukaryotes have multiple linear chromosomes of varying sizes. For the specific purpose of decreasing their DNA size to ensure fitting inside a cell, prokaryotes employ DNA supercoiling (Ma & Wang 2016).

However, because eukaryotes have much more DNA than prokaryotes (3234 mega-base pairs vs. 4.4 mega-base pairs), they need to utilize a more complex strategy to position their DNA, which, if stretched from end to end, would be two meters long, properly inside a microscopic cellular space (O'Donnell *et al.,* 2013). Specifically, this is done by sequential levels of coiling, starting with DNA wrapping around histone proteins forming a structure known as a nucleosome, then nucleosomes coiling to form chromatin fibers, and then chromatin further condensing into densely packed chromosomes (Simpson *et al.,* 2023).

Molecular level of DNA

A molecule of DNA is made up of two long polynucleotide chains consisting of subunits known as nucleotides. A nucleotide comprises a nitrogenous base, a pentose sugar, and at least one phosphate group (Figure 4a) (Ghannam *et al.,* 2022). In the case of DNA, the sugar is 2'-deoxyribose, and thus it has no hydroxyl group attached to its 2' (pronounced "two prime") carbon; this is in contrast to ribose sugar in RNA, which does not have the 2' position of its pentose sugar to be reduced (or deoxygenated). A phosphate group covalently binds to the 5' carbon of 2'-deoxyribose. Since the 2'-deoxyribose and the phosphate group are always present, the nitrogenous bases they incorporate distinguish the four DNA nucleotides (Travers & Muskhelishvili 2015).

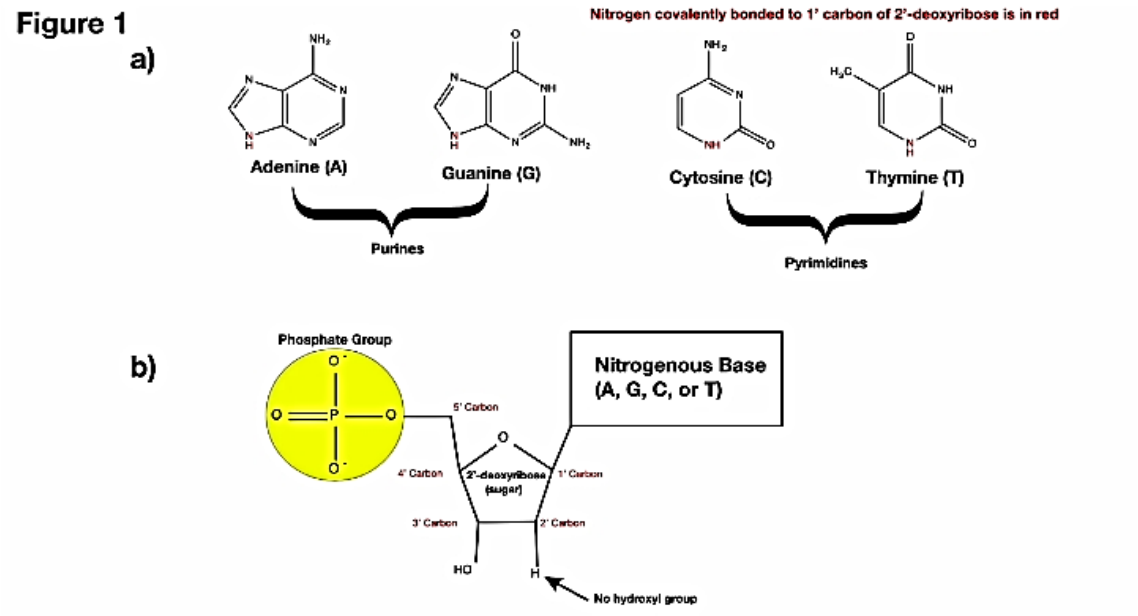


Figure 4: DNA structure; **Source:** (Ghannam *et al.,* 2022)

A nucleotide can incorporate four main nitrogenous bases, two of which are purines and two that are pyrimidines (Figure 4b). Both purines and pyrimidines are heterocyclic aromatic compounds, as they contain nitrogen atoms in their carbon-based ring, which are essential for the hydrogen bonding that holds the two strands of the DNA molecule together. However, while pyrimidines are six-membered rings, purines consist of a five-membered ring fused to a six-membered ring. The two pyrimidines found in DNA are thymine (T) and cytosine (C), while the two purines are Adenine (A) and Guanine (G). The purines and pyrimidines differ slightly in structure, but their functional groups are attached to the same basic heterocyclic form. These nitrogenous bases are covalently bonded via a nitrogen atom to the 1' carbon of the deoxyribose sugar in a nucleotide (figure 4).

Although four major nitrogenous bases make up the nucleotides of DNA, other uncommon non-primary or modified bases have been found to exist in nature (Kumar, 2018). The most common modified bases in bacterial genomes are 5-methylcytosine, N6-methyladenine, and N4-methylcytosine. These modifications have been shown to protect DNA from restriction enzymes, which cleave DNA at specific sites. In all eukaryotic genomes, the most common modified base is 5-methylcytosine which is critical in regulating gene expression (Liyanage, 2014).

Each strand of DNA is made up of a string of nucleotide subunits linked at their sugar moieties (figure 5a). Specifically, nucleotides in a DNA strand are bound together via ester bonds between the phosphate group attached to their 5' carbon and the hydroxyl group on the 3' carbon of an adjacent nucleotide. This bond is known as a phosphodiester bond, and it forms via a condensation reaction during DNA synthesis. As a result, each strand of a DNA molecule has a series of nucleotides with their 5' phosphate and 3' hydroxyl group participating in phosphodiester bonds. Each strand of a eukaryotic DNA molecule has a "free" 5' phosphate group on one end, not bonded to a hydroxyl group, and a "free" 3' hydroxyl group on the other end, not bonded to a phosphate group. This asymmetry has led to the adoption of the convention where DNA is read in a particular direction, from its 5' end to its 3' end (Ghannam *et al.,* 2022). The sequence of nucleotides that make up a molecule of DNA is referred to as its primary structure (Lehman, 1974).

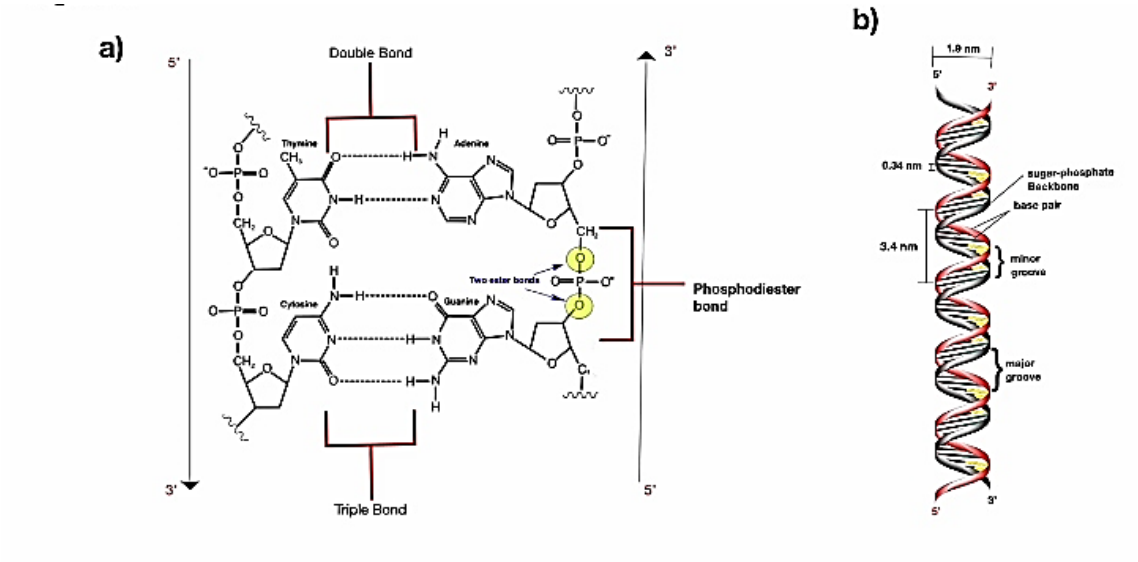


Figure 5: DNA nucleotide subunit **Source:** (Ghannam *et al.,* 2022)

A DNA molecule consists of two chains of polymerized nucleotides running side-by-side, joined together by hydrogen bonds forming between their nitrogenous bases (figure 5). Notably, the nucleotides bond in a particular fashion, with A pairing with T and G pairing with C; A and T pairing is by two hydrogen bonds, and C and G by three. These specific pairings result in about a 1 to 1 ratio of pyrimidines and purines in any given cell, a concept known as Chargaff's rule. This pairing scheme is called complementary base-pairing and is the most energetically favorable pairing possible. DNA is structured so that the sugars of

each strand are on the outside, while the bases hydrogen bond on the inside, resulting in what is known as the sugar-phosphate backbone. Thus, two chains of sugar-phosphate backbones run side-by-side with complementary paired nitrogenous bases hydrogen bonding between them. Notably, the two strands of a DNA molecule run in an antiparallel fashion so that the 5' end of one strand is the 3' end of the other (Szabat, 2015). This base pairing of nucleotides between the two strands of a single DNA molecule is called DNA's secondary structure.

The three-dimensional shape of a DNA molecule, or its tertiary structure, is a right-handed double helix (Figure 5). The hydrogen-bonded bases on each strand are stacked in parallel and run perpendicular to the sugar-phosphate backbone. As indicated by its x-ray diffraction pattern, the bases are regularly spaced at 0.34 nm apart along the axis of the helix (Damaschun *et al.,* 1983). Additionally, there are about ten pairs of bases per turn, as a complete turn of the helix is made every 3.4 nm. DNA has a +36-degree rotation per base pair (bp) and a helical diameter of 1.9 nm (Damaschun *et al.,* 1983). When focusing on the backbone of the DNA helix, two helical grooves exist with different widths, known as the minor and major grooves (Figure 5b). The minor groove describes the space between the two antiparallel DNA strands that run closest together, while the major groove describes the space where they are furthest apart. These specific dimensions describe the B form of DNA, the major form present in most stretches of DNA in a cell (Chaires *et al.,* 1982) This is in contrast to DNA's much rarer A and Z forms. The A form is a right-handed double helix with less distance between the bases (0.256 nm), and thus more bases per turn (11 bp per turn) and a smaller helical rotation per base pair (+33 degrees). Z DNA is a left-handed double helix and is most present in the human genome, where many purines and pyrimidines are alternating in succession (i.e., in a sequence such as GCGCGCGCGCG). DNA primarily takes the B form, in contrast to any other form, because it is the most energetically stable tertiary structure (Bacolla *et al.,* 2013).

A notable property of DNA is the ease of reversible separation of its two strands due to hydrogen bonds being relatively weak compared to covalent bonds. This is important because fundamental cellular processes such as DNA replication and RNA transcription rely on proteins accessing individually separated strands of DNA. Thus, during these processes, proteins known as helicases move down the DNA molecule and unwind the two strands by disrupting the hydrogen bonding between bases. However, when the cellular processes requiring strand separation are complete, the complementary strands can easily

re-anneal. This property of reversible separation can be experimentally induced via the heating and cooling of a DNA molecule and is referred to as denaturation or "melting" (Ekundayo & Bleichert 2019).

One notable structural phenomenon of DNA tertiary structure is supercoiling, or the coiling of the larger, already coiled DNA molecule. Specifically, in a DNA molecule that has its ends fixed, such as in the circular DNA found in prokaryotes or the smaller DNA segments that make up a larger chromosome in eukaryotes, separation of the individual strands of DNA during cellular processes causes the DNA to twist-up past the points of strand separation, leading to strain on the larger DNA structure (Ma & Wang 2016). This transient over-winding of the larger DNA structure when separating individual strands is known as positive supercoiling. Every cell has enzymes that keep DNA actively underwound to compensate for this, resulting in perpetual negative supercoiling, where the larger DNA structure coils in a left-handed fashion. This results in the strands of DNA needing less energy to be separated and keeps the molecule primed for easy separation in the events of transcription and DNA replication.

Function of DNA

The unique structure of DNA is ultimately responsible for its function as being the material that stores and transmits genetic information from one generation to the next. Specifically, the four nitrogenous bases that comprise the sequence of nucleotides in a DNA molecule enable an enormous amount of information stored in minimal space. DNA's sugar-phosphate backbone and helical structure make it more stable, less prone to damage, and more compact; however, the hydrogen bonds that hold the strands of DNA together make it more accessible for its biological functions as they are individually weak but cumulatively strong. Also, the complementary base pairing of nucleotides in DNA enables accurate semiconservative replication as each strand carries identical genetic information and serves as an independent template during DNA replication (Travers & Muskhelishvili 2015; Chaudhry & Khaddour 2023).

DNA modification

DNA modification, also referred to as genetic engineering or genetic modification, is defined as the alteration of the genetic makeup or original genome of organisms that wouldn't occur by natural means (Excedr, 2022).

Genetic modification wouldn't come about until 1973, when American biochemists Stanley N. Cohen and Herbert W. Boyer invented recombinant-DNA technology and subsequently revolutionized the way scientists look at DNA.

Genetic modification has allowed researchers to edit (adding, deleting, or changing the nucleotides from the DNA sequence) the genomic DNA of organisms by using different biotechnology methods, including recombinant DNA, gene targeting, or genome editing.

The terms "engineering" and "modification" are interchangeable. Both are used in the context of labeling genetically modified, or "GMO", food. Broadly, genetic engineering denotes selective breeding, cloning, or stem cell research (Excedr, 2022).

A popular method of DNA modification includes the making of a recombinant plasmid, the steps of which are listed below:

- Step 1: Selecting the desired gene and isolating it using restriction enzymes.
- Step 2: Amplify it using Polymerase Chain Reaction (PCR).
- Step 3: Insert the desired gene into bacterial plasmids using restriction enzymes and DNA ligase.
- Step 4: Plasmids are transfected into competent bacteria, selected, and allowed to multiply. Once cloned, the gene can be easily isolated and analyzed/modified.
- Step 5: Cloned or modified DNA can be introduced back into the donor organism.

Application of DNA Modification

It has several applications in a range of areas, including medicine, research, industry, and agriculture which are briefly discussed below:

Application in Medicine

The three major applications of DNA modification in medicine are (Excedr. 2022):

- drug manufacturing
- creating transgenic model animals that mimic human conditions

- gene therapy

For example, mouse hybridomas have been adapted through genetic engineering to create human monoclonal antibodies. Further, gene therapy, developed by editing the human genome, is used in several clinical applications, including X-linked SCID, Parkinson's disease, and cancer.

Application in Research

Genetic modifications are popular for engineering biochemical pathways, studying the function of specific genes, and discovering the location of specific gene expression. Moreover, in labs, the technique is used extensively on animals for its efficacy in treating human diseases.

Application in Industrial

The process involves the transformation of organisms' cells with a gene involved in the synthesis of a useful protein. Then, the transformed cell or organism (that can involve any bacteria, yeast, insect cell, or mammalian cells) is placed inside a bioreactor for the mass production of the specific protein.

Application in Agriculture

DNA modification is an essential technique to create genetically modified crops or livestock. It's a solution to make crops and livestock resistant to several diseases and pests while also enhancing their productivity.

DNA Modification Due to Epigenetic Changes

The process of DNA modification also involves epigenetic changes that have heritable effects (especially when the modification occurs in germline cells) on gene expression without changes in DNA sequences.

These changes in organisms are regulated by DNA modifications, histone modifications, and non-histone chromosomal protein modifications that control several gene functions.

The most common chemical modifications of histones include enzymatic methylation catalyzed by the DNA methyltransferases (DNMTs) (Jin *et al.,* 2011), acetylation catalyzed by Histone deacetylases (HDACs) (Seto & Yoshida 2014), phosphorylation catalyzed by T4 polynucleotide kinase (Camden *et al.,* 2019)., ubiquitination, and sumoylation. They play

essential biological roles in epigenetic regulations. Most of them indicate DNA damages and are involved in DNA repair pathways.

Methylation of DNA

DNA methylation is a heritable epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases (DNMTs) (Robertson, 2005). The methylation or demethylation of histones is one of the most extensively studied epigenetic mechanisms. Depending on the position or base modification, it can cause the activation or silencing of the transcription. For example, in vivo, histone H3 trimethylation at lysine 4 (H3K4me3) activates the transcriptional process that recruits DNA repair enzymes. However, DNA methylation at the fifth position of cytosine (5mC) in CpG dinucleotides (cytosine nucleotide followed by a guanine nucleotide), causes gene silencing. These methylations are carried out by DNA methyltransferases (Jin *et al.,* 2011).

Cytosine Methylation at CpG sites is the most common genetic modification that has a crucial role in epigenomics, gene expression, or other essential functions of organisms. Some non-CpG methylation where guanine is replaced by adenine, cytosine, and thymine has also been observed in undifferentiated human embryonic stem cells (hESCs) (Excedr, 2022).

The methylation patterns at different sites are executed by the enzyme DNA methyltransferase (DNMT), encoded by DNMT mRNA after the replication process. The enzyme catalyzes the transfer of a methyl group from S-adenosyl methionine to DNA.

5-hydroxymethylcytosine (5hmC), a product of the ten-eleven translocation (TET) in the demethylation of 5mC, is another in vivo DNA modification. The molecule further undergoes the oxidation process, leading to the formation of 5-formylcytosine (5fC) and 5-carboxycytosine (5CaC).

5hmC is considered an essential epigenetic factor in many fields, including stem cell renewal, cancer development and progression, and neurological disorders.

There are different available techniques to detect nucleic acid modifications. The copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC), coupled with click DNA labeling, enables genome-wide profiling of any DNA modification at single-base resolutions or locus-specific resolutions.

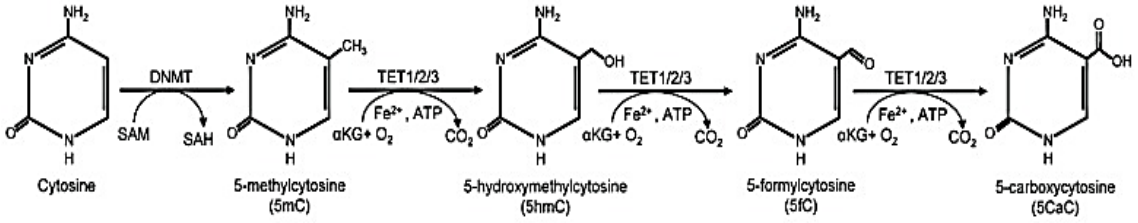


Figure 6: Illustration of DNA methylation **Source: (**Liyanage *et al.,* 2014)

The above illustrates the generation of DNA methyl modifications. Cytosines are converted to 5-methylcytosine (5mC) by DNA methyltransferase (DNMT) enzymes through transfer of a methyl group from S-adenosyl methionine (SAM). Ten Eleven Translocation (TET) enzymes catalyze the oxidation of 5mC to 5-Hydroxymethylcytosine (5hmC) through a chemical reaction which involves alpha-ketoglutarate (αKG), Oxygen (O2), Adenosine triphosphate (ATP) and Fe2+. Similar reactions further oxidize 5hmC into 5-formylcytosine (5fC) and 5-carboxycytosine (5CaC).

Histone modification

Histones undergo a variety of posttranslational modifications, including acetylation, methylation, phosphorylation, and ubiquitination, that lead to changes in chromatin structure with consequences for gene expression (Kouzarides, 2007). The "histone code" hypothesis suggests that different types and combinations of modifications differentially alter chromatin structure and transcriptional potential (Jenuwein & Allis 2001). Acetylation of the ε-amino group of lysine residues in the aminoterminal tails of histones H3 and H4 is the best-characterized histone modification most consistently demonstrated to promote transcription. Histone acetylation is catalyzed by histone acetyl transferases, which are recruited to acetylation sites via requisite transcriptional cofactors, such as cyclic AMP response element-binding protein and p300 (Berndsen & Denu 2008). Histone deacetylation is associated with CpG methylation and inactive chromatin structure. Four classes of HDACs catalyze deacetylation, and they are themselves regulated by posttranslational modification (Mellert & McMahon 2009). Histone lysine methylation represents another important class of histone modification that alters gene expression, although the determinants of expression (repression or promotion) are complex; they

depend on the position of the lysine and the extent of its methylation and are not fully elucidated as yet. Similar to histone acetylation, histone methylation is readily reversible, with many known histone lysine methyltransferases and demethylases that target specific lysines and specific mono-, di-, or trimethylation states. Figure 1 depicts the relationships between DNA methylation and common histone modifications in epigenetic regulation of gene expression.

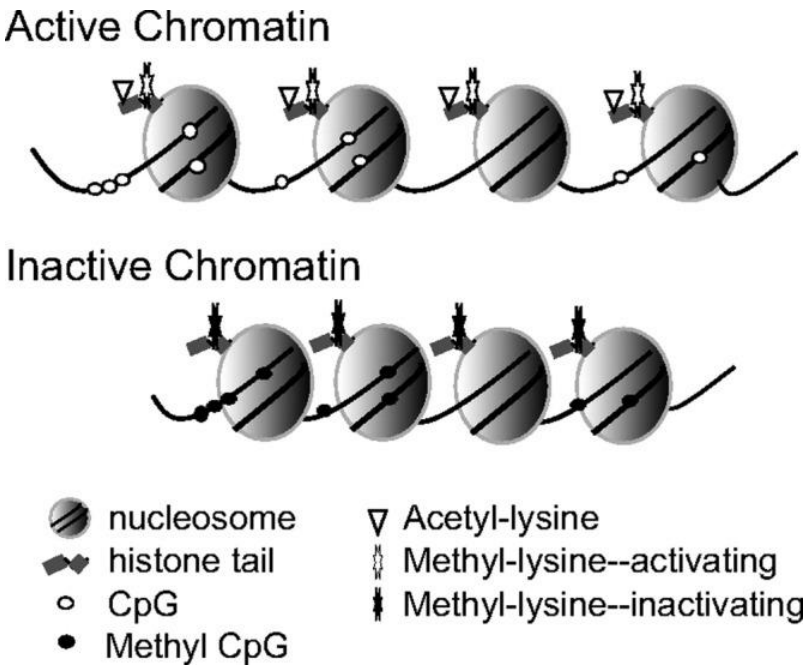


Figure 7: Chromatin structure and epigenetic tags.

Source: (Loscalzo & Handy 2014).

Nucleosomes are indicated as spheres, with sites of methylation and acetylation on DNA and histone proteins listed in the key (reprinted with permission from reference.

DNA modification for drug tartgetting

DNA molecules have shown potential for drug delivery owing to their predictability and programmability. Various chemotherapeutic drugs and nucleic acid drugs have been developed to combat different diseases. Systemic delivery of these drugs is challenging because of these existing limiting factors, such as solubility, stability, targeting transportation, membrane penetration, and controllable release. Numerous versatile nanocarriers, including liposomes, polymers, and inorganic nanoparticles, have been constructed. Considering the possible cytotoxicity and immunogenicity of these carriers,

the wide application of these carriers is limited. Fortunately, various chemical modifications of nucleic acids can be employed to construct versatile chemically modified DNA nanostructures (Wang, *etal.,* 2022).

Due to the continuous development of DNA nanotechnology and the unique properties of DNA nanomaterials, many drug delivery systems based on stimuli-responsive DNA nanostructures have emerged (Wang, Liu, Wu, Lou, & Liu, 2022). Drug targeting facilitates the drug to reach its appropriate site so as to show the desired pharmacological response as per the therapeutic need. A clear understanding of the level of targeting makes it easy to decide the suitable targeting moiety, ligand, or carrier system (Rojo, Sousa-Herves, & Mascaraque, 2017).

Mechanism of DNA Targeting

Some examples and mechanism of the commonly used methods of DNA modifications for drug targeting purposes are seen below:

Gene Editing using CRISPR-Cas9:

CRISPR-Cas9 is a revolutionary gene editing tool that enables precise modifications in the DNA sequence **(**Jayachandran *et al.,* 2022). This technique enables scientists to add, delete, or modify specific genes or gene segments associated with diseases. The mechanism involves the following steps:

1. CRISPR/Cas9 complex formation
2. CRISPR/Cas9 complex attaches to the target DNA sequence and induces a double-strand break (DSB) at the specific site
3. The next step is the insertion of donor DNA and results in the transformed DNA sequence

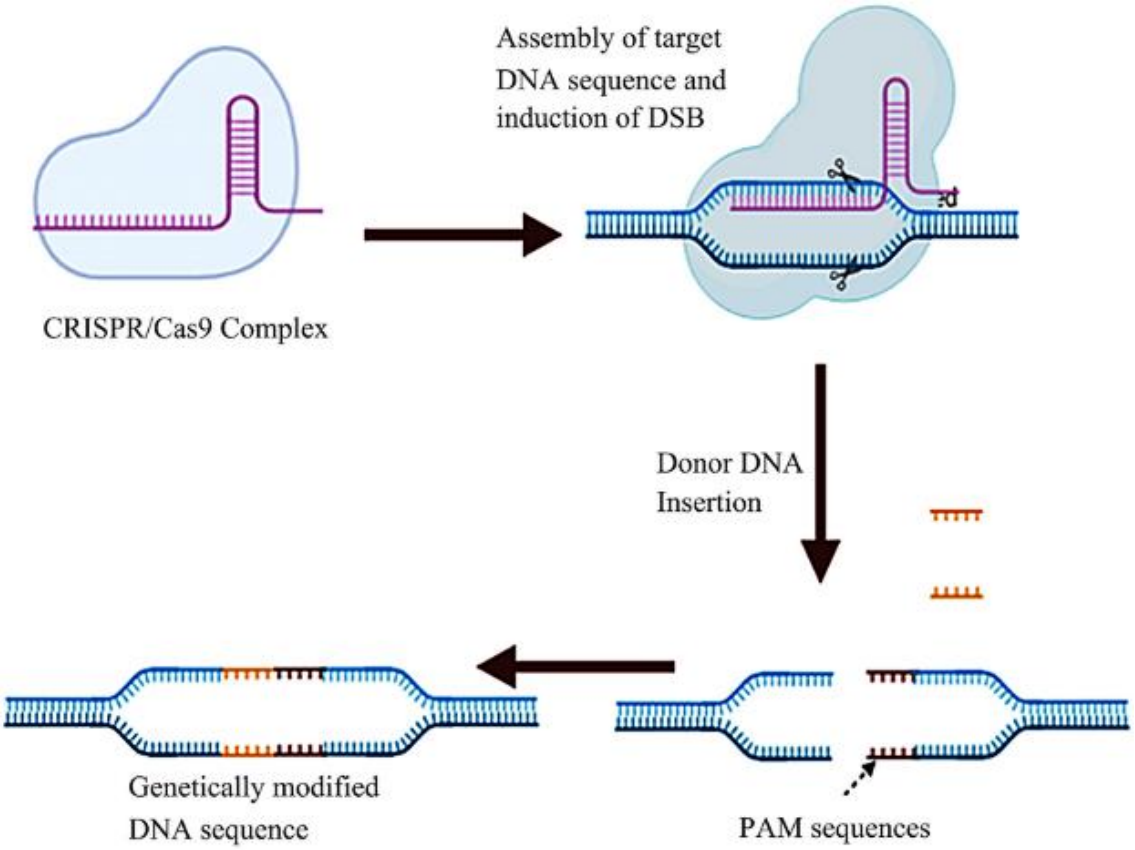


Figure 8: mechanism of CRISPR/Cas9

Source: (Jayachandran *et al.,* 2022)

Antisense Oligonucleotides (ASOs)

ASOs are short, synthetic nucleic acid molecules that specifically bind to the target DNA or RNA sequence. The mechanism (figure 2) act by either causing (1) RNA cleavage or (2) RNA blockage.

RNA cleavage mechanism

- (1a) RNase H1 mediated cleavage,
- (1b) RNA interference (RNAi)
- (1a) ASO-mRNA heteroduplex recruits RNase H1 enzyme and this enzyme cleaves the target mRNA
- (1b) mRNA degradation by siRNA associated with RNA inducing silencing complex (RISC).

RNA blockage

- (2a) Steric hindrance
- (2b) Splice modulation
- (2a) ASO-mRNA complex sterically blocks and prevents the interaction of mRNA with ribosomes for protein translation.
- (2b) is an example of splice switching oligonucleotides (SSO).

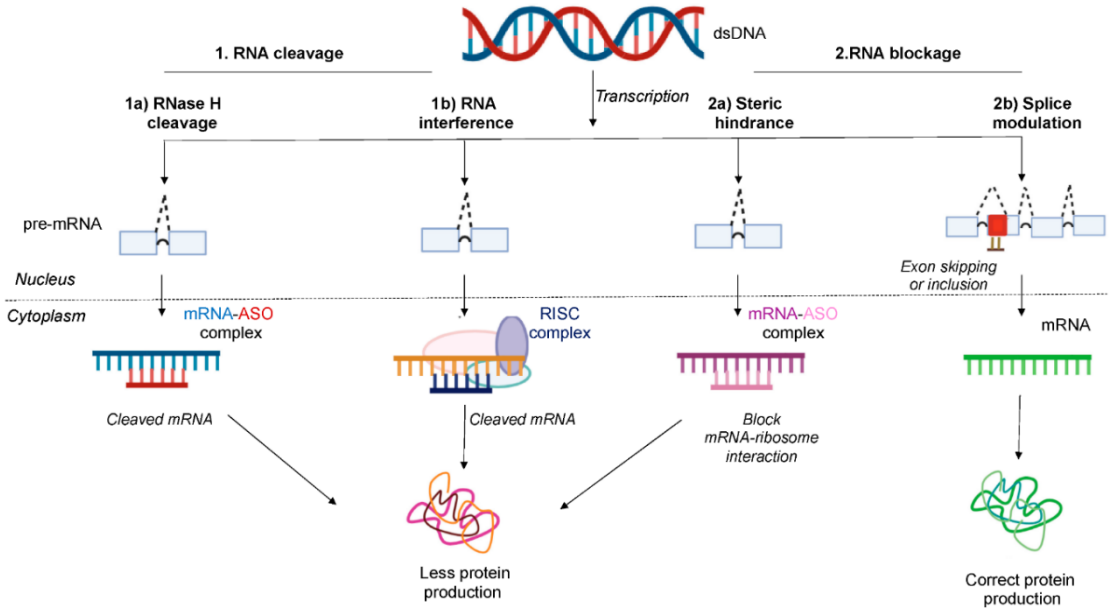


Figure 9: mechanism of Antisense Oligonucleotides (ASOs) Source: (Dhuri et al., 2020)

- Rectangles depict the coding exon regions separated by a curve depicting the non-coding intron region of the pre-mRNA.
- The red square represents the mutated region of the exon.
- The dashed line represents the splicing pattern of pre-mRNA.

Epigenetic Modifications:

Epigenetic modifications involve chemical changes to the DNA or histone proteins associated with DNA. These modifications can regulate gene expression without altering the DNA sequence. Examples include DNA methylation and histone modifications. The mechanism involves:

a. DNA methylation: Addition of a methyl group to the DNA molecule, typically at cytosine residues, by DNA methyltransferase enzymes. Methylation can repress gene expression.

b. b. Histone modifications: Chemical modifications, such as acetylation, methylation, phosphorylation, or ubiquitination, occur on histone proteins. These modifications can affect the accessibility of DNA and influence gene expression.

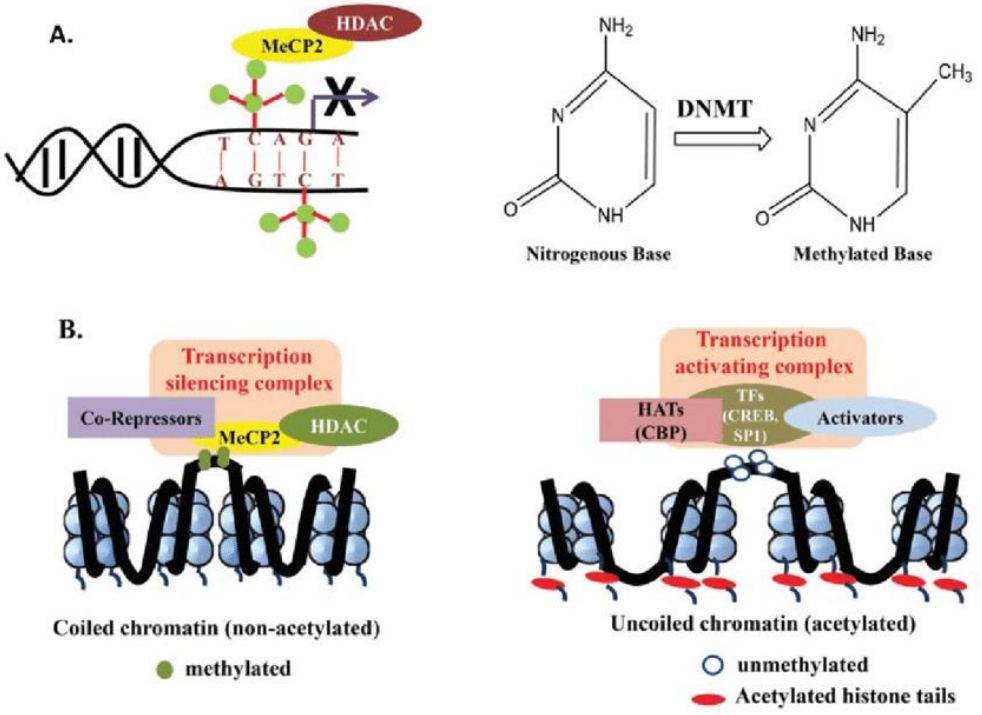


Figure 10: mechanism of Epigenetic Modifications; Source: Monga et al., 2016)

DNA methylation (A) and histone deacetylation (B, left panel) will promote chromatin coiling and inhibit the binding of transcriptional machinery; while histone acetylation (B, right panel) will facilitate uncoiling of chromatin and enhance binding of the transcriptional machinery.

Targeted Drug Delivery:

DNA modifications can also be used to facilitate targeted drug delivery. By modifying specific DNA sequences, researchers can design drug carriers or nanoparticles that are specifically recognized and internalized by cells expressing complementary DNA or RNA

sequences. This approach enables precise drug delivery to target tissues or cells, enhancing therapeutic efficacy and reducing off-target effects.

Plant nanoparticles for targeted drug delivery:

Plant nanoparticles, such as exosomes and virus-like particles (VLPs), can be utilized as carriers to deliver modified DNA payloads to target cells or tissues for therapeutic purposes. The concept of using plant nanoparticles for DNA modification and drug targeting includes: Engineering Plant Nanoparticles, Encapsulation of Modified DNA and Targeted Delivery.

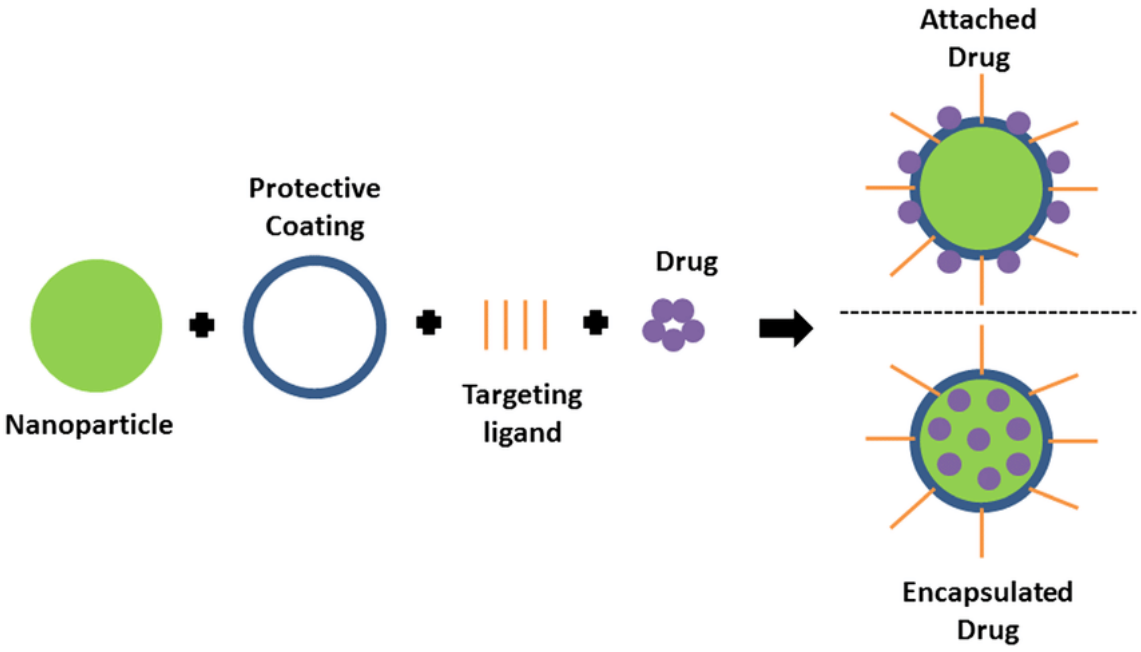


Figure 11: Plant nanoparticles for targeted drug delivery; Source: (Mcnamara & Tofail, 2017).

Conclusion

Over the past decades, different chemical modifications of DNA have been developed and successfully employed for various biomedical applications. DNA modification for drug targeting purposes holds great potential for advancing precision medicine and targeted therapeutics. Various strategies can be employed to modify DNA sequences, including gene editing techniques, targeted demethylation, and DNA methylation as a therapeutic target. These modifications can be achieved using plant nanoparticles, such as exosomes

and virus-like particles (VLPs), which offer unique advantages in terms of biocompatibility, scalability, and targeted delivery. DNA modification allows for precise manipulation of genetic information, enabling correction of disease-causing mutations, suppression of disease-promoting genes, or targeted expression of therapeutic genes.

Recommendation

Very little information can be found on this topic; therefore, it is recommended that more research be done on this study. With more knowledge on the topic this could bring about more new ways in which DNA can be modified for drug targeting purposes.

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