Describe the key features of the three-dimensional structure of the B-form of DNA. Discuss other three-dimensional structures that DNA may adopt and how these structures may affect its biological function.

Deoxyribonucleic acid (DNA) is a macromolecule essential to all forms of life - it holds genetic information which codes for proteins required for growth and development (Nguyen, 2018). Discovery of DNA occurred in 1869 when Frederic Miescher extracted a 'nuclein' from a nucleus which was found to be isolated DNA with its associated proteins still attached (Dahm, 2005). Over decades, research was conducted by scientists, including Rosalind Franklin, who worked on crystallography of DNA fibres. She produced the first image of the DNA helix and observed that DNA properties change in anhydrous conditions – later found to be A-DNA. In 1953, Watson and Crick built on Franklin's research and proposed that DNA has a three-dimensional double helix structure (Figure 1) (Tyson, 2003). Though there are three major forms of DNA, research over time has proven that there is a total of 21 possible forms (Bansal, 2003).

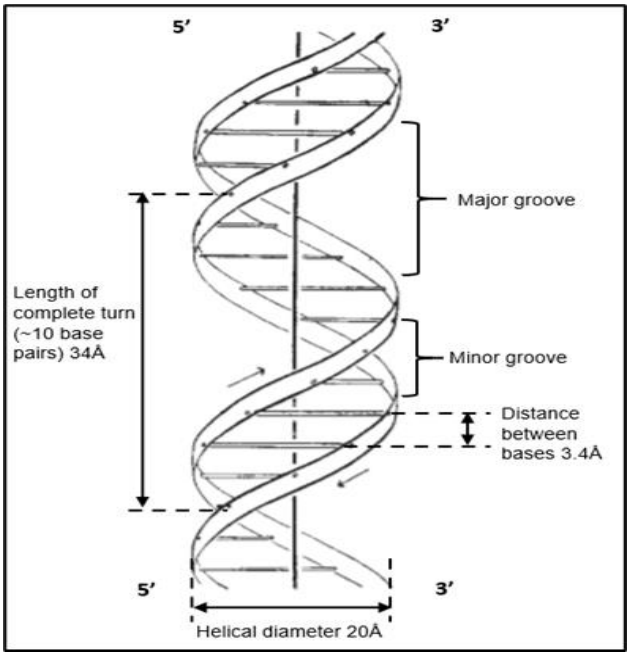

Figure 1. Labelled Watson-Crick model of DNA. *This figure displays a simplified diagram of the proposed double-helix structure by Watson and Crick in 1953. The two coiling strands represent the two sugar phosphate polynucleotide chains (in the 5'-3' direction) while the horizontal rods represent base pairs bonded together by hydrogen bonds which hold the two strands together. There is a distance of 3.4Å between each base pair. The major and minor grooves have been labelled on the diagram. The arrows along the strands represent the direction of the strands - B-DNA is right-handed. (Figure 1 has been adapted from original source (Watson and Crick, 1953) and labelled by Seroczynska W).*

The Watson-Crick model, called B-DNA, summaries that DNA has a right-handed double helix structure consisting of two complementary polynucleotides connected by hydrogen bonds between nitrogenous bases on both strands (Alberts *et al*, 2002). Each chain contains repeating nucleotides, joined together by phosphodiester bonds. As shown in Figure 2(a), a nucleotide is a 5-carbon pentose sugar with a nitrogenous base and a phosphate group attached at carbon-1 and carbon-5 respectfully. Phosphodiester bonds form between the phosphate group of one nucleotide and the hydroxyl of

another, resulting in formation of a polynucleotide chain (Figure 2(b)) called the sugar phosphate backbone - making up the primary structure of DNA and providing initial structural stability. Due to phosphodiester bonding, the 5' end of the strand (the start) will have a free phosphate group and the 3' end (the end) will have a free hydroxyl group - resulting in polarity of the strand (Schwabe, 2020).

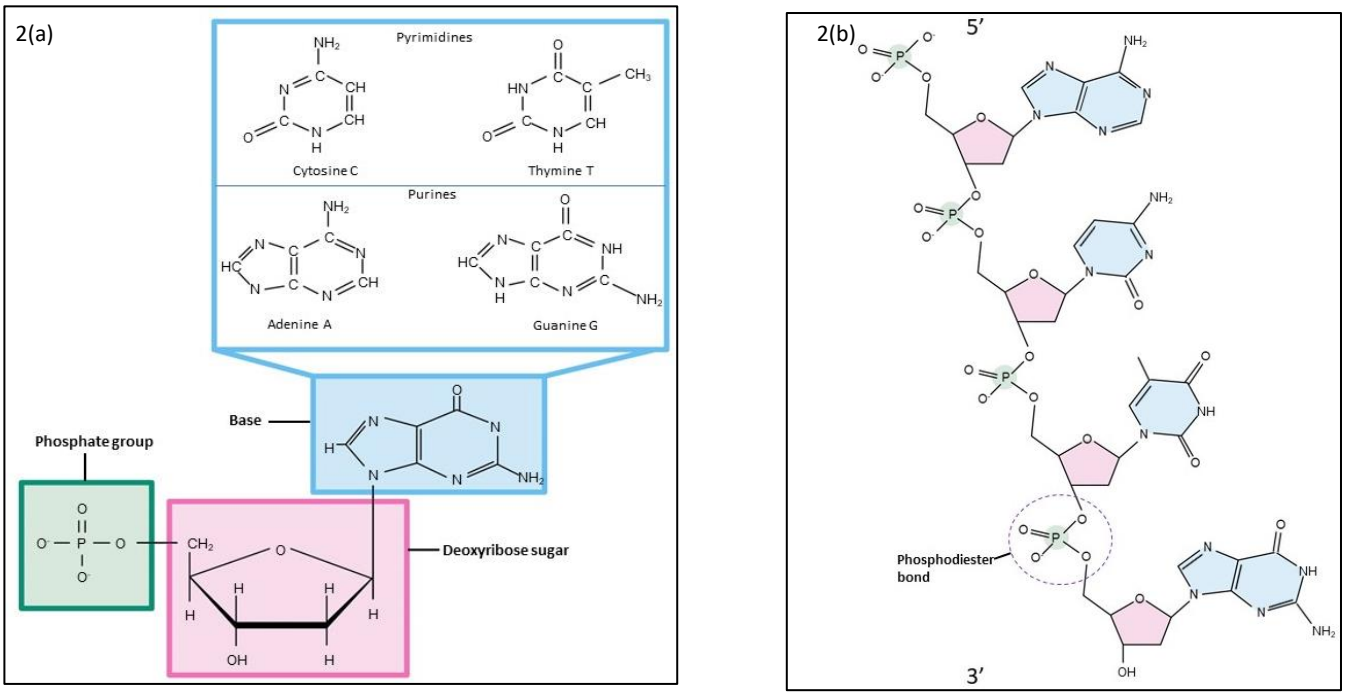

Figure 2. Nucleotides and polynucleotide formation. *This figure displays the overall structure of nucleotides and a polynucleotide chain.* ***Figure 2(a):*** *This displays the structural formula of a nucleotide. The pink box highlights a 5-carbon deoxyribose sugar in a furanose structure. The carbon atoms have been labelled. On carbon-5 there is a phosphate group (green box) attached to the sugar via esterification. Carbon-1 is bonded to a nitrogenous base, in this figure the example given is guanine (blue box). All four bases that bind with the sugar are shown above the nucleotide, including their full structure and classification (purine or pyrimidine).* ***Figure 2(b):*** *This part shows the complete structure of one polynucleotide strand in the 5' to 3' direction. A phosphodiester bond (labelled) forms between each nucleotide. 5' end has a free phosphate group and 3' end has a free hydroxyl group. (Figure 2 has been adapted from source* (*Discovery of the structure of DNA.* n.d.) *and illustrated by Seroczynska W).*

The secondary structure of DNA relies on hydrogen bonding between bases in order to maintain a stable interior of the helix and join the strands together. The bases involved are adenine (A), thymine (T), cytosine (C) and guanine (G), which are classified into purines (A and G) with a two-ring structure and pyrimidines (C and T) with a single-ring structure (Figure 2(a)). Purines are complementary to pyrimidines, more specifically, A pairs with T via two hydrogen bonds and G pairs with C via three hydrogen bonds, this is called base specificity (Alberts *et al*, 2002). Watson and Crick based this concept on Chargaff's rule that concentration of pyrimidines is equal to concentration of purines. Generally, hydrogen bonds are weak, this allows or easier strand separation during DNA replication. However, collectively they are strong enough to stabilize the helix. The more G-C interactions in the DNA, the more stable the helix, as a G-C pair has more hydrogen bonds compared to A-T (Jeffrey and Saenger, 1994). Base pairs have a similar width (which keeps the stands at an equal distance apart) and a flat planar structure therefore they can stack on top of each other at a distance of 3.4 Angstroms (Å) (Aryal, 2019) - this forms the tertiary structure of DNA. Van der Waals forces are present between stacked bases adding significant stability to the overall structure. Furthermore, to maximize base pair stacking, the strands coil around each other - called a plectonemic coil, measuring 20Å in diameter. In one helical turn are 10.4 base pairs measuring 34Å in length. Due to base pair specificity, the strands cannot run parallel to each other as the bases would not pair up correctly (Potaman and Sinden, 2013). Hence, one strand remains in the 5'-3' direction while the other flips to the 3'-5' direction, this way polarity is neutralised and therefore the strands are antiparallel to each other (Schwabe, 2020). Despite, if DNA were to be flipped, the sequence would remain the same, as shown in Figure 3.

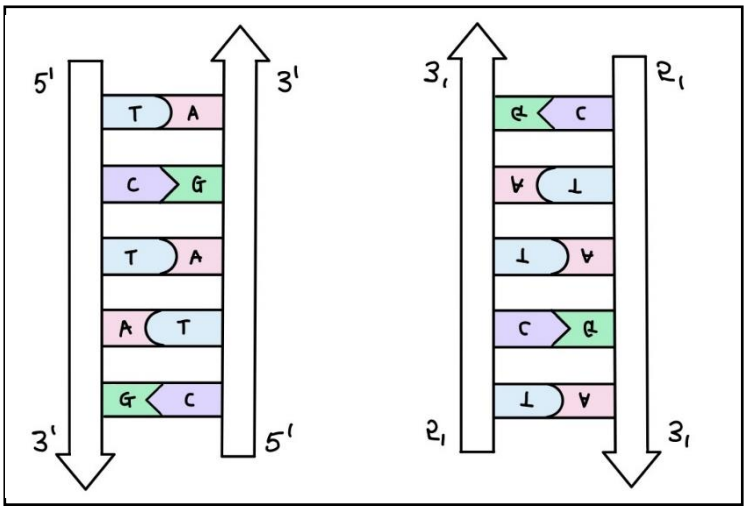

Figure 3. Reading DNA sequences. *On the left, a five base pair sequence is shown, written in the 5'-3' direction. The right-hand side image is the same image as on the left but flipped vertically. This figure shows that the DNA sequence does not change when it is viewed from a different perspective, this is because DNA is always read in the 5' to 3' direction. And so, the left sequence reads TCTAG and the right sequence also reads TCTAG (Seroczynska, unpublished).*

Moreover, due to the polar nature of phosphate ions, DNA is negatively charged. Cells have a high humidity and therefore there is a high abundance of positive oxygen atoms resulting in electrostatic repulsion between the DNA and the water. As a result of this, the negatively charged polynucleotide strands remain on the outside, creating a hydrophilic surface, shielding the hydrophobic base pairs from interaction with water.

Another key feature of the three-dimensional structure of B-DNA is the formation and sizing of grooves. Major and minor grooves are formed because glycosidic bonds between the bases and the backbone are not directly opposite those on the partner strand, this also gives DNA an asymmetrical structure. In B-DNA, major grooves are wide and deep allowing proteins to bind and read the base sequence (or alter the structure) through the major groove without having to enter the DNA helix. Minor grooves are

narrow and deep; thinness of the minor groove exposes only the edges of the base pairs, which is enough for the protein to identify the sequence, so structural stability is unaffected (Berg, Tymoczko and Stryer, 2002).

Summarising the tertiary structure of DNA, it refers to folding of the strands and packing of bases into a specific three-dimensional structure. The tertiary structure determines the overall structural form of DNA, most predominant examples being B-form, A-form and Z-form. There are four variables by which DNA structure may differ: handedness, helix turn length, base pair number per turn and groove sizes (Table 1) (Cahill *et al*, 2012).

	B-form DNA	***A-form DNA***	***Z-form DNA***
Helical sense	Right-handed	Right-handed	Left-handed
Diameter	20A	26A	18A
Repeating unit	1bp	1bp	2bp
Major groove	Deep and wide	Deep and narrow	Flat / non-existent
Minor groove	Deep and narrow	Shallow and wide	Deep and wide
Base tilt	6	20	7
Bp per helical turn	10.5	11	12
Bp contact distance	3.4 Å	2.9 Å	7.4 Å
Helix rise per bp	3.4 Å	3.6 Å	3.7 Å
Sugar pucker conformation	c-3' endo	c-2'endo	c-2' in pyrimidines; c-3' in purines
Glycosyl bond conformation	anti	anti	anti for pyrimidines; syn in purines

Table 1. Comparisons between three main forms of DNA. *This table compares the main features and specifics of B-DNA, A-DNA and Z-DNA. The columns are presented in the following order: Features, B-form DNA, A-form DNA, Z-form DNA. The abbreviation 'bp' stands for base pair(s). (Table 1 has been adapted from sources (Chu, 2020; Freeman, 2008)).*

A-DNA was discovered by Franklin via x-ray crystallography, she observed that under dehydrated conditions and low humidity, B-DNA conforms into A-DNA (Elkin, 2003). A-DNA is also a right-handed double-helix with two anti-parallel strands. This form has 11 base pairs per helical turn measuring 2.9Å

in length. More base pairs per turn means the twist angle will be smaller, hence a more compact and shorter structure compared to B-DNA (Figure 4). Base pairs are at a 20° angle and therefore the major groove is narrow and deep meaning that proteins will be unable to bind to the DNA and retrieve specific sequences, however a wider and shallow minor groove will allow access to some basic information (Potaman and Sinden, 2013). Different electrostatic forces within A-DNA and B-DNA result in varying abilities to withstand diverse cellular conditions. While B-DNA is able to function in standard physiological and diversified conditions, A-DNA is only observed in highly saline conditions and once cell humidity falls below 75% (Jose and Porschke, 2004). A-DNA is able to withstand extreme bacterial conditions, for instance, in sporulating bacteria, a protein binds to DNA initiating conformation from B-DNA to A-DNA, this change in structure protects the bacteria from external stimuli such as UV radiation, high pressures and heat (Potaman and Sinden, 2013).

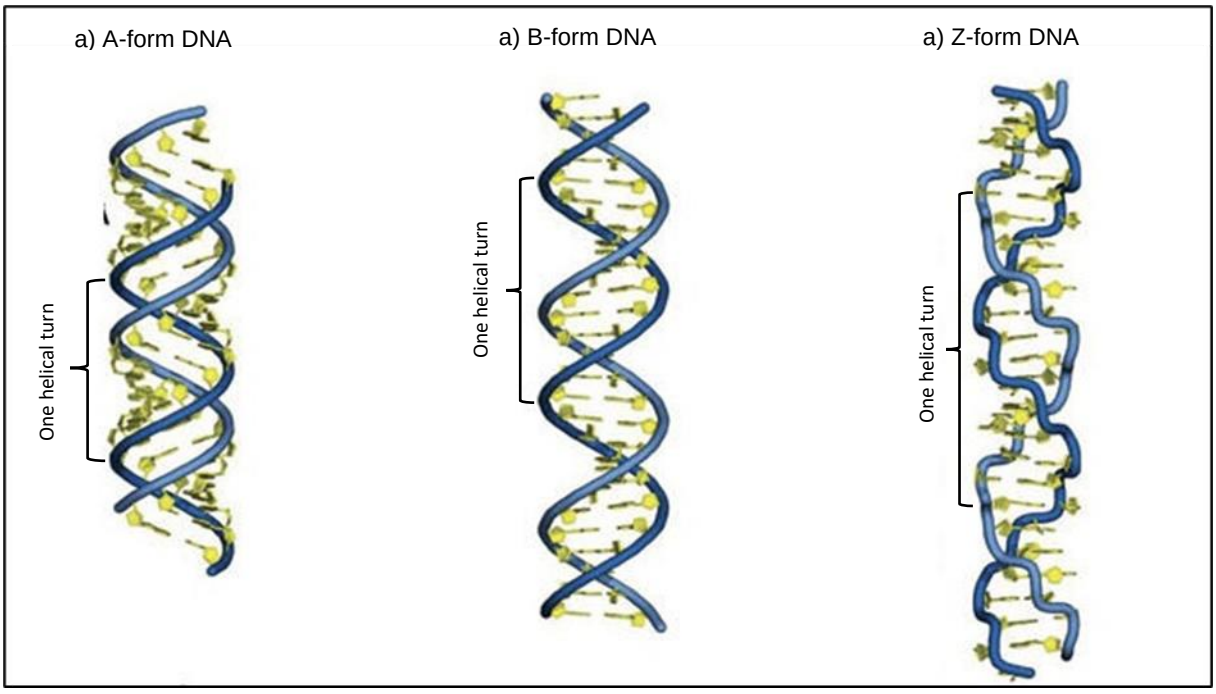

Figure 4. Forms of DNA. *This figure displays the 3D structures of the main forms of DNA. Nitrogenous base pairs are indicated in yellow and the two sugar phosphate strands are in blue.* ***(a):*** *A-form DNA has a compact right-handed structure compared to the other two forms. Base pairs are at a 20° angle to the plane.* ***(b):*** *B-form DNA is displayed in the middle, it follows the Watson-Crick model and is also right-handed, with bases perpendicular to the axis.* ***(c):*** *Z-DNA, which adopts a left-handed helix and a zig-zag pattern. (Figure 4 has been adapted from original source* (DNA, 2018).

The third main form is Z-DNA which adopts a left-handed helical structure. The strands are made up of polynucleotides with two repeating units, unlike one in A- and B-DNA, hence forming a zigzag patterned coil (Figure 4). The units are composed of alternating purine-pyrimidine bases (GpC step), such as (GC)n, which are very prone to conformational changes compared to pyrimidine-purine (CpG step) pairs. In Z-DNA purines are rotated into the syn conformation while the pyrimidines maintain the anti-conformation (found also in B-DNA and A-DNA (Table 1)). The total turn angle of the helix is 60° as the

GpC step is 9° and the CpG step is 51°. In one turn there are 12 base pairs (length of 4.6Å) and a base pair contact distance of 7.4Å. Z-DNA has a flat major groove and a deep minor groove (Ravichandran, Subramani and Kim, 2019; Stefanov Yury, 2008). Wahl and Rich discovered that Z-DNA forms behind an RNA molecule moving through the helix during transcription, and it is stabilised by negative supercoiling – this is important as Z-DNA is present in high salt conditions, thus supercoiling balances the electrostatic forces between salt ions in the cell and the negative phosphate groups. The exact function of Z-DNA is unclear however due to its presence at the start of a gene, Rich and his team suggested that Z-DNA may have a regulatory role within gene expression. Another potential role in vivo is inhibition of DNA modification by proteins as transformation into Z-DNA would prevent B-DNA specific endonucleases from modifying the base sequence (Herbert, 2005).

E-DNA is another form - a stable intermediate formed during B-DNA to A-DNA conformation. Similarly, to B-DNA, base pairs are planar, but the grooves are like those in A-DNA due to 3'-endo sugar conformation. Research suggests that E-DNA may serve a role in base pair sequence mutation (Vargason, 2011).

Due to being the most researched DNA form, it is a common misconception that the DNA structure proposed by Watson and Crick (B-DNA) is the most important molecule for biological processes. In fact, without the varying structural differences between all the forms of DNA, some processes would not be able to take place and many organisms such as bacteria would not be able to exist. In simple terms, all these different structural forms of DNA are evolutionary adaptations to varying cellular conditions for the molecule to be able maintain its ability to hold and protect genetic information.

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