Molecular Mechanisms for the B–Z Transition in the Example of Poly[d(G–C)·d(G–C)] Polymers. A Critical Review

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Contents

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1. Introduction

One of the most important achievements in our understanding of the biochemistry of DNA is our awareness that the double helix has considerable conformational flexibility. The concept of structural flexibility was illustrated by the discovery of left-handed DNA and other DNA conformations (e.g., A-DNA, C-DNA, etc.). In the 1970s, the development of DNA synthesis has made it possible to carry out crystal X-ray diffraction studies that would prove the structure. In 1978 $d(C-G)_3$ was synthesized and crystallized and diffraction patterns were obtained which revealed a left-handed double helix with two antiparallel chains held together by Watson−Crick base-pairs (Table 1).[1] In the left-handed DNA conformation every other base rotated around the glycosyl bonds so that the bases alternated in *anti*- and *syn*-conformations along the chain. The puckering of sugars was C3′-*endo* for purine residues and C2′-*endo* for pyrimidine residues. The stacking patterns of GpC and CpG sequences were drastically different.[2] The zigzag arrangement of the backbone lead to the name Z-DNA for the new DNA conformation (Figure 1). The relationship between the Z-DNA and the more familiar right-handed B-DNA structure was evident from an experiment that showed that the far-UV circular dichroism of poly[d(G−C)·d(G−C)] inverted in 4 M NaCl solution.[3] That the inversion was due to a conversion from B-DNA to Z-DNA was established by examining the Raman spectra of these solutions and the Z-DNA crystals.[4] Left-handed Z-DNA is characterized not only by the left-handed twist of the double helix but also by an alternating *anti-syn* configuration of its base pairs. Because purines adopt the *syn*-conformation more readily than pyrimidines, Z-DNA formation is favored in sequences with alternations of purines and pyrimidines.[2,5−8] The importance of synthetic DNA as poly[d(G−C)·d(G−C)] regarding the B−Z transition is due to the fact that it is a highly stable sequence and, historically, the B−Z transition was first observed with this alternating purine−pyrimidine polymer in high-salt solutions.[3] Interestingly, the methylated analogue poly[d(G−m^5C)·d(G−m^5C)] undergoes the B−Z transition even in low-salt solutions[9] and is the most stable duplex against thermal denaturation.[10]

A short introduction about the state-of-the-art in the Z-DNA field requires a brief incursion on a relevant aspect: the biology of Z-DNA. Does Z-DNA have a biological role? Today, the question raised by Alexander Rich[11] has yet a difficult answer although there are many indicators that point toward an important role of left-handed Z-DNA in a variety of cellular functions *in vivo*. The search for a definite

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biological role for Z-DNA gradually gained approval[12] although the existence of this structure was initially received with skepticism. Since the elucidation of the structure of Z-DNA, the question of the biological role of this conformation has remained in the forefront of the research in the field. Thus, even though the central problem of modern molecular biology is the elucidation of the mechanisms of gene regulation, an important question to answer would be the possible relevance that this process has in the B−Z transition. Thus, a good number of reports have dealt with the question concerning the "long road from left-handed Z-DNA structure to biological function", for instance, (i) the first indication that Z-DNA could exist *in vivo*;[13] (ii) the observation that the Z-DNA binding domain of ADAR1 enzyme binds to both Z-RNA and Z-DNA;[14] (iii) the finding that antibodies to Z-DNA bind preferentially to actively transcribed genes;[15−18] (iv) the very strong correlation of the ability to undergo the B−Z transition with the GC content of the DNA sequence[19,20] (the meaningful link of the B−Z transition with GC content is shown by the coupling of the positive spike of GC content near the gene transcription start site and the negative spike near the stop site);[21] and (v) the strong connection between the B−Z transition and the gene expression, supported by the observation that gene GC content correlates with the level and among-tissue breadth of gene transcription.[19,22] A time line referring to the biological role of left-handed Z-DNA has been recently reviewed.[23]

Despite the dense literature about the biological role of the left-handed Z-DNA, the molecular mechanisms, which drive the B−Z transition, have remained obscured and contrasting results have appeared in the literature on this

Table 1. Comparison of Helices of B-Form and a Variety of Z-DNA Forms

parameter[a]	B-form[b]	Z-form[c]	Z[WC]-form[b]	Zf-form[d]
helical sense	right-handed	left-handed	left-handed	left-handed
helix diameter	18.4	16.0	18.2	19.0
base pairs per turn	10	12	12	12
helical twist[e]	36	−60	−60	−60
helix pitch (rise per turn)	34.0	44.6	44.6	43.5
mean rise per base pair	3.40	3.72	3.72	3.63
phosphorus radius:				
inner	9.2	6.1	5.7	8.5
outer	9.1	8.0	9.1	9.5
mean base inclination	15.0	2.3	8.1	5.0
sugar pucker				
G	C2′-*endo*	C3′-*endo*	C2′-*endo*	
C	C2′-*endo*	C2′-*endo*	C3′-*endo*	
glycosidic bond				
G	*anti*-	*syn*-	*syn*-	*syn*-
C	*anti*-	*anti*-	*anti*-	*anti*-
P−P distance across minor groove				
GpC to CpG (width)	11.8			
GpC to GpC (max.)		18.1	13.3	
CpG to CpG (min)		8.6	5.7	

[a] Distances in angstroms and angles in degrees. [b] Data from ref 82. [c] Data from ref 1. [d] Data from ref 149. [e] The helical twist is per base pair in the B-form and per dinucleotide in the Z-form of DNA, respectively.

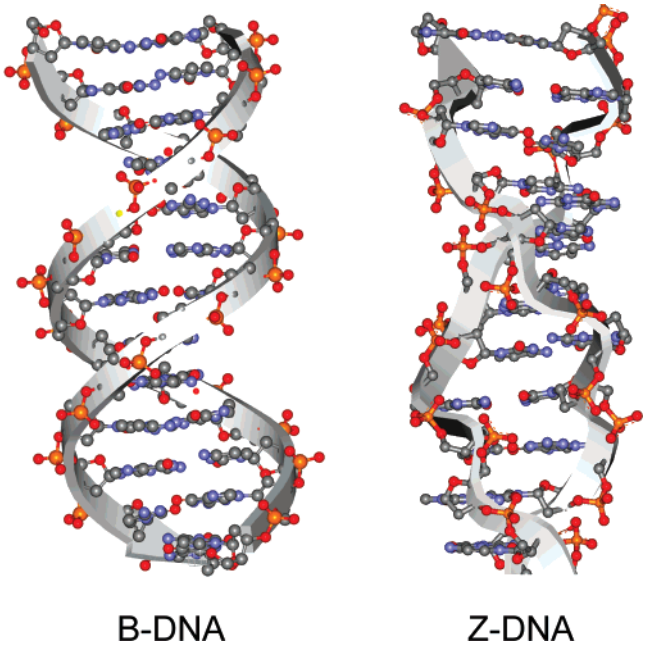


Figure 1. Side views of B-DNA and Z-DNA. The irregularity in the Z-DNA backbone is illustrated by the ribbons which go from phosphate to phosphate residues along the DNA chain following a zigzag discontinuous left-handed course.

subject. Thus, the chemical−biological community has constantly ignored the analysis of the molecular mechanisms leading to Z-DNA. The present survey is concerned with the molecular mechanisms for the B−Z transition as proposed in the last 30 years and the consequent progression of ideas. Discussion of our current perception of the mechanisms proposed is limited to this evolutionary context. However, by drawing upon this assembly of information, attempts to expand this critical argument and to describe in detail most of the predictions that can be deduced from a "probable" unified model will be made.

As far as we know, this review gives an updated time line of the B−Z transition models, which have been proposed from both chemical and physicochemical points of view.

2. The B- to Z-DNA Transition

Because of the possible involvement of Z-DNA in gene expression,[2] both the Z-DNA and the B−Z transition have been subjects of intense study.[24] This foreword will help to follow the development of this field of research through its time line course. Now, and after briefly introducing the readers to the B−Z transition and to its stabilizing factors, we will go further into describing a catalog of key experiments that any molecular model of the transition should be able to meet.

2.1. Description of the B–Z Transition and Stabilizing Factors

The conversion of B-DNA to Z-DNA is associated with a "flipping over" of the base pairs so that they are upside down in their orientation relative to what would be found in B-DNA. The flipping over resulted in both the production of a *syn*-conformation in every other base (Figure 2) and a change in the deoxyribose-ring pucker in alternate bases. The result of the reorganization was that the phosphate groups were closer together in Z-DNA than in B-DNA (Figure 3). The equilibrium between B-DNA and Z-DNA conformations is determined mainly by three factors: (i) environmental conditions, (ii) chemical structure of the polymers, and (iii) degree of topological stress generated by supercoiling.

The importance of the environmental conditions was evident from the classical experiment that showed the inversion of the far-UV circular dichroism spectrum of poly-[d(G−C)·d(G−C)] in a 4 M NaCl solution.[3] In the presence of a solution with a high-salt concentration, the electrostatic repulsion of the phosphate residues decreases, stabilizing the Z-DNA conformation. Of particular interest is the discovery that oligonucleotides with the alternating purine−pyrimidine sequence adopt the B-DNA conformation at high water activity but adopt the Z-DNA conformation at low water activity,[1,25,26] a finding that could explain the high cooperativity of the transition. In any case, the salt concentration is only one factor among many others that modify the environmental conditions of DNA (e.g., the solvent structure, which has been suggested to play a critical role in defining

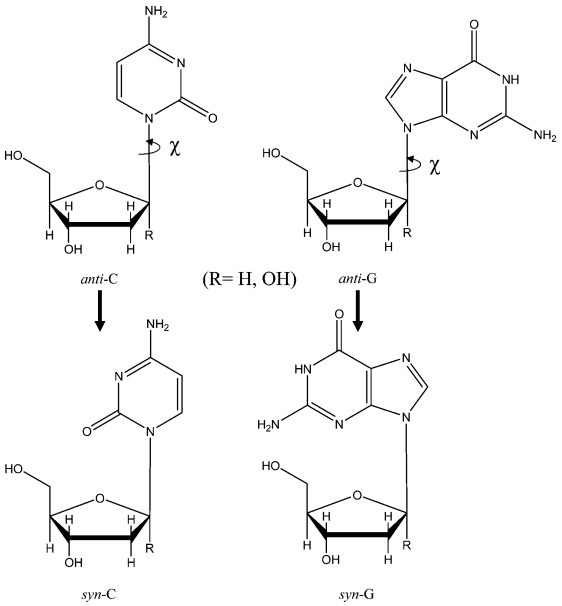


Figure 2. Sterically allowed orientations of C and G bases with respect to their attached ribose units. In B-DNA, the nucleotide residues all have the *anti*-conformation. In Z-DNA, the nucleotide residues acquire the *anti*-conformation for pyrimidines and the *syn*-conformation for purines. The 2′-R (R = H, OH) has been considered to include RNA, which can also adopt the Z-DNA form.

the conformation of polynucleotides,[27,28] the type of counterion present in the solution,[9,28,29] the temperature,[30,31] the pressure,[32] and the presence of other molecules in the solution, such as, for example, peptides,[33] drugs including traditional coordination complexes of Pt(II) and Co(III) (see Table 2),[34,35] and also recently reported metal complexes such as $[Ni(H_2O)_6]^{2+}$, Cu(II), and Zn(II) complexes containing C(15) substituted macrocyclic pentaaza ligands.[36,37]).

Relative to the chemical structure of the polymer it is necessary to bear in mind that since purines adopt the *syn*-conformation more readily than pyrimidines, the Z-DNA formation is favored in sequences with alternations of purines and pyrimidines, especially alternations of C and G[2], although other base alternations are possible.[5−8] On the other hand, several studies have showed that chemical modifications such as, for instance, the cytosine methylation were able both to destabilize the B-conformation[38] and to stabilize the Z-conformation of DNA in synthetic poly[d(G−m^5C)·d(G−m^5C)] polynucleotides.[9] Other substitutions such as iodine, bromine, and aza at the C5 position of cytosine, or the phosphorothioate modification of the d(GpC) linkage,[39] also stabilize the Z-conformation of DNA. The most common covalent modifications of the polymer has been reviewed elsewhere.[2]

Finally, the demonstration that Z-DNA could be formed under conditions of topological stress was the critical step to assign a possible biological role to the Z-DNA molecule.[40] It is known that the advancing polymerase generates positive supercoils in the DNA template ahead of it and negative supercoils behind it[41] and that the level of Z-DNA in metabolically active, permeabilized mammalian cell nuclei is regulated by torsional strain.[42] The influence of topoisomerase-I and gyrase mutations on the stability of left-handed Z-DNA was investigated, and the results found indicate that a variety of factors, such as protein−DNA interactions, activity of topoisomerases, and the resulting supercoil density, contribute to the B to Z transition inside living cells.[43] Thus, Z-DNA would be a higher-energy

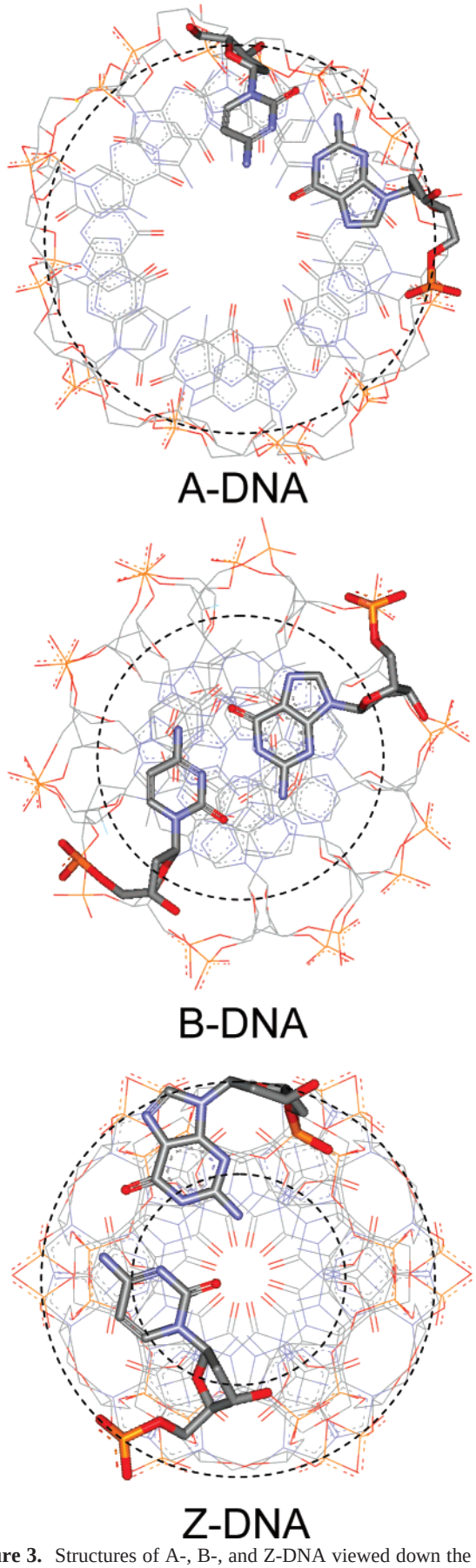
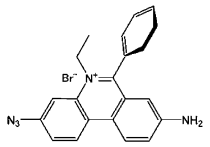
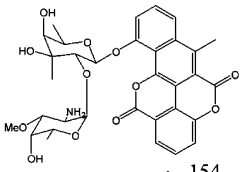
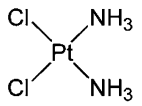
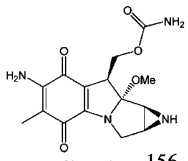
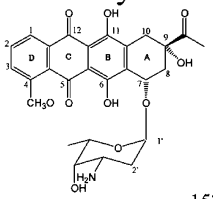
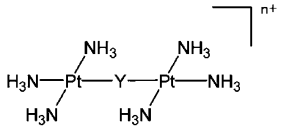
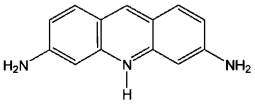
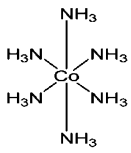
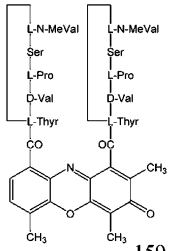
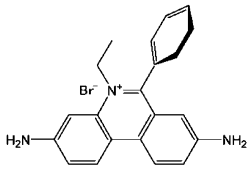
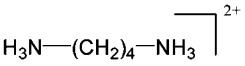
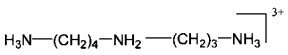
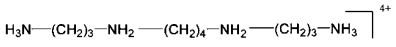
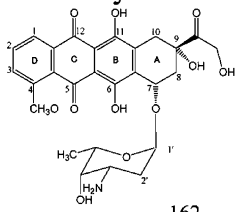
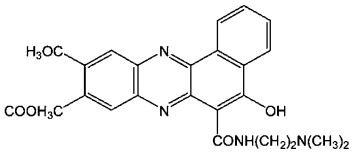
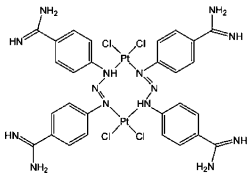
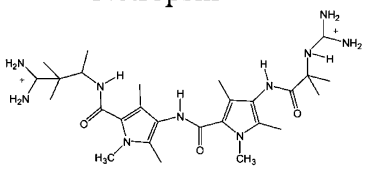


Figure 3. Structures of A-, B-, and Z-DNA viewed down the helix axis. The nearest base pair is drawn with sticks, and the ribose ring atoms are crossed by a circle. The Protein Data Bank codes for the structures are as follows: (A-DNA)[145] 2D47; (B-DNA)[169] 1BNA; (Z-DNA)[170] 3ZNA.

Table 2. Some Inducers of Both B−Z and Z−B Transitions and Inhibitors of the B−Z Transition in d(G−C)·d(G−C) Polymers

B-Z Inducers	B-Z Inhibitors	Z-B inducers
$(K\text{-}X)_n\text{-}K$ peptide[33,45] K=Lysine X=non-bulky aminoacid residue	Ethidium monoazide[150] 	Elsamicin A[66] 
†*cis*-DDP[151] 	‡Mitomycin C[152,153] 	Daunomycin[154] 
Polynuclear Pt-complexes[155] 	Proflavine[156] 	KWGK peptide[157] K=Lysine W=Tryptophan G=Glycine K=Lysine
Cobalt hexamine[9] 	Actinomycin D[156] 	Ethidium bromide[158] 
POLYAMINES[35] Putrescine  Spermidine  Spermine 	‡Adriamycin[159,160] 	*NC-182[161] 
	Pt-berenil[162] 	‡Netropsin[26,163] 

† *cis*-Diamminedichloroplatinum(II). ‡ Netropsin and mitomycin C behave as both B−Z inhibitors and Z−B inducers. * Antitumor compound having a benzo[*a*] phenazine ring.

conformation than B-DNA and will be only formed in torsionally stressed plasmids, being stabilized by negative supercoiling. On the other hand, the DNA supercoiling in the chromatin structure is related with the gene expression.[44]

An extensive list of chemical factors that stabilize the B−Z transition influencing the B−Z equilibrium of poly[d(G−C)·d(G−C)] polymers has been previously reviewed.[2] Thus, such factors can be summarized as (i) covalent modifications of G and/or C, (ii) ionic changes in solution, (iii) solvent modifications, (iv) small molecule effectors, and, lately, (v) the binding of peptides and proteins.[33,45]

2.2. B–Z Junctions

Since enzyme-mediated denaturation of a localized segment of DNA is a prerequisite to DNA replication or transcription, it is of interest to determine the stability of DNA oligomers containing unusual structures because they may act as recognition sites for DNA binding proteins. It is

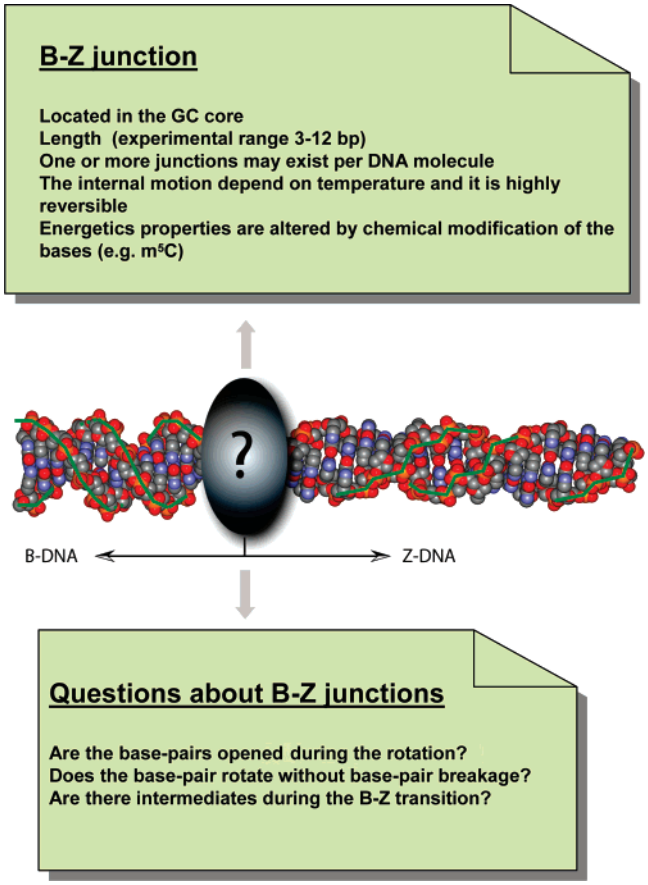


Figure 4. van der Waals side views of B-DNA and Z-DNA. The B−Z junction is indicated by an ellipse with a question mark inside. The panel located on top of the figure describes some experimental observations relative to a B−Z junction. The one at the bottom of the figure raises certain questions that must be answered experimentally.

accepted that two or more conformations may exist within the same DNA molecule, thereby generating conformational junctions due to stereochemical considerations (e.g., B−A DNA,[46] B−C DNA,[47] B−Z DNA,[40] etc.). The first example of the presence of B−Z junctions between right-handed B-DNA and left-handed Z-DNA conformations was detected in plasmids.[40] A characterization of a B−Z junction in a model system as a short DNA oligonucleotide was carried out later.[48] There are a number of parameters that are necessary for description of B−Z junctions, such as (i) the number of base pairs implicated in the junction (the length of the junction), (ii) the chemical modification of the bases in the junction, and (iii) factors that affect the internal motion of the junction (Figure 4).

There is no agreement regarding the length of the junction. The lengths of the B−Z junctions ranged from 10−12 base pairs long[49] to 3 base pairs long.[50,51] Table 3 shows some experiments carried out to give an idea about the length of a B−Z junction. It is important to take into consideration the chemical modification of the bases in the junction, such as the methylation of cytosine in the C5, which alters the structural and energetic properties of the B−Z junction.[38,52,53] Finally, relative to the internal motion of the junction, NMR saturation-transfer experiments have provided evidence which suggests that the junction's internal motion is temperature dependent.[50] It is important to point out that in all cases where a stable B−Z junction is formed it is located within the GC core of the molecules and not within the flanking segments.[54] Such a structural predisposition is due to the fact that alternating $d(C-G)_n$ sequences can adopt both B- and Z-conformations and may sustain the structural deformation.

However, a question remains in the forefront of the B−Z junction research field that relates its internal structure with the molecular mechanism of the B−Z transition. Are the hydrogen bonds of one or more base pairs broken in the junction or not, as occurs in a helix−coil transition? So, if the base pairs remain intact, then the stacking interaction between two or more base pairs would change to account for stereochemical requirements.[3]

2.3. Key Experimental Observations

A molecular model for the B−Z transition should be able to explain key experimental data reported in the literature and also be capable of predicting results that, at present, lack experimental evidence. As a general approximation to the problem, we will divide the compilation of information into two sections.

The first section will comprise a catalog of experiments related with typical parameters of the B−Z transition, such as the cooperativity, the counterion concentration present in the polymer solution, the temperature of the solution, the rate of the B−Z transition, the type of counterion used in the experiment, the length of the polynucleotide, the degree of the transition, etc. The above parameters will be useful to describe the results found in the experiments.

Serious contradictions have been found when comparing to each other the results obtained from some of these experiments. For instance, it was initially reported that the B−Z transition of the polymer was entropically driven.[3,9] However, it was shown lately that an important enthalpic contribution accounted for the B−Z transition.[55−57] On the other hand, it was revealed[58] that the rate constant of the B−Z transition decreases when increasing the length of the polymer. However, most recently, data indicate that the rate constant increases with increasing the length of the poly-

Table 3. Measurements of the Length of a B−Z Junction in Diverse Polynucleotides by Using Several Physicochemical Techniques

technique	DNA[a]	length (base pairs)	refs
supercoiling	pRW751 containing $d(G-C)_{16}$ and $d(G-C)_{13}$ inserts	10−11	107, 164, 165
supercoiling	plasmid containing $d(G-C)_n$ inserts	4−8	166
CD	sized poly[d(G−C)·d(G−C)]	4−6	49
	sized poly[d(G−m^5C)·d(G−m^5C)]	10−12	49
ORD, CD	poly[d(G−C)·d(G−C)]	5	3
NMR, IR	d(CGm^5CGCGXACATGT)[b]	4−8	167
P NMR	d(Gm^5CGm^5CGm^5CGm^5CACTGACTG)[c]	4−6	48
NMR, CD	d(Gm^5CGm^5CGm^5CGm^5CACTGACTG)	3	50
Raman spectroscopy	d(CGCGCGCGCGCGAAAA)	≤3	51
	d(CGCGCGAAAAA)	≤3	51

[a] The deoxyoligonucleotides are indicated by the 3′−5′ sequence, and their complements are omitted for simplicity. [b] X = 1-cyano-2-dioxy-β-D-erythropentofuranose. [c] m^5C indicates 5-methyldeoxycytidine.

mer.[49,59] As can be noticed, these results are clearly in contrast. In any case, it is important to take into account that one of the primary unsolved problems in the B−Z transition lies in the reversal of the direction of the 5′−3′ progression in the backbone chains.[1]

The second section describes and summarizes the data obtained from experiments on the B−Z transition that have not yet found a satisfactory explanation although some steps have been taken in the appropriate direction. For example, there are experiments reported in the literature that establish that at low salt concentration the more stable form of the synthetic double-stranded polymer poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is the Z-DNA conformation, in contrast with the results of classical experiments described before. Thus, the existence of a double transition like the Z ↔ B ↔ Z transition as a function of the counterion concentration in poly(dG-m^{5}dC)·poly(dG-m^{5}dC) has been described.[60] Such an experiment has also been reported in the absence of any oligovalent cation in the solution.[61] To explain these contrasting results, an experiment was designed which indicated that at low counterion concentration a Z-DNA form of poly[d(G−m^{5}C)·d(G−m^{5}C)] could be stabilized by trace amounts of divalent cations (Mg^{2+}, Ca^{2+}, etc.) present in solution. Thus, only when such cations had been removed and monovalent sodium was present at low concentration would the polymer adopt the classical B-DNA conformation.[62] However, the low counterion concentration of the Z-DNA form of poly[d(G−m^{5}C)·d(G−m^{5}C)] resulted to be very stable and the temperature was unable to drive the Z−B transition at any temperature although at 100 °C the Z-DNA polymer melted into single strands.[63] Another experiment described that upon a monotonic increase of the ionic strength, the well-established B- to Z-DNA transition of $d(C-G)_8$ is followed by a second conformational change leading from Z-DNA back into a right-handed B-like form.[64] A similar Z−B transition has also been observed induced by temperature at high ionic strength in poly[d(G−C)·d(G−C)][65] or also induced by chemical products[66] (Table 2). These observations indicated, in contrast with the current convention, that the Z motif represents an unstable configuration relative to the B form at both *low* and *high* salt concentrations.

Thus, despite the existence of numerous models to account for the B−Z transition, experiments have not yet come up with a definite reason for these observations. Specifically, most Z-DNA models fail to provide a clear understanding of why the reversible B−Z transition is facilitated when the transition path is blocked by drug-induced bulky adducts situated in the mayor groove of DNA[2,39,67] or why a certain purine−pyrimidine repeat does not yield a left-handed helix under the adequate conditions while another does.[2,39,67−69] Furthermore, the hydrogen exchange displayed by amino groups in GC base pairs is an order of magnitude slower in a left-handed helix than in a right-handed helix[70,71] of the same sequence, which does not have a satisfactory explanation in the currently proposed models.

3. Summary of Models Proposed for the B–Z Transition

Extensive studies of the left-handed Z-DNA structure have been carried out since the determination of its structure by Rich and co-workers.[2,72,73] Despite the existence of various models to account for the B−Z transition, experimental data have not yet come up with a credible response to the structural and dynamical features of the B−Z transition. At present (Figure 5A) there are different views regarding the mechanism of the B−Z transition. One view is that the B−Z transition involves base-pair opening before rotation (see Figure 5B).[1,3,58,59,74−77] The second view is that the transition involves no disruptive transfiguration through a series of correlated internal motions in the backbone and glycosyl bond to facilitate base-pair rotation without base-pair breakage (Figure 5C).[78−81] Unfortunately, a problem remains unsolved in a number of models: they involve a winding of 180° rotation of bases and reorganization of the backbone that create a steric dilemma known as the *chain sense paradox* (see Figure 6).[82,83] Some authors even tried to address this paradox by suggesting that the left-handed helix observed in crystallography is not the Z-DNA with its chain sense reversed but an alternative left-handed version whose chain sense is the same as that of Watson−Crick B-DNA. This alternative form of DNA has been named Z[WC]-DNA.[82] Experimental data obtained recently are in agreement with this last statement. In fact, the experimental findings show that the handedness of the DNA duplex can be reversed by breaking one base pair and extruding the bases from the duplex.[84] To solve this dilemma, other models have been proposed. One view is that the transition occurs through an intermediate A-DNA type conformation with no disruption of interbase hydrogen bonds and without severe sterical impediments. The more recent proposed model that involves an intermediate, the S-DNA, during the B−Z transition is

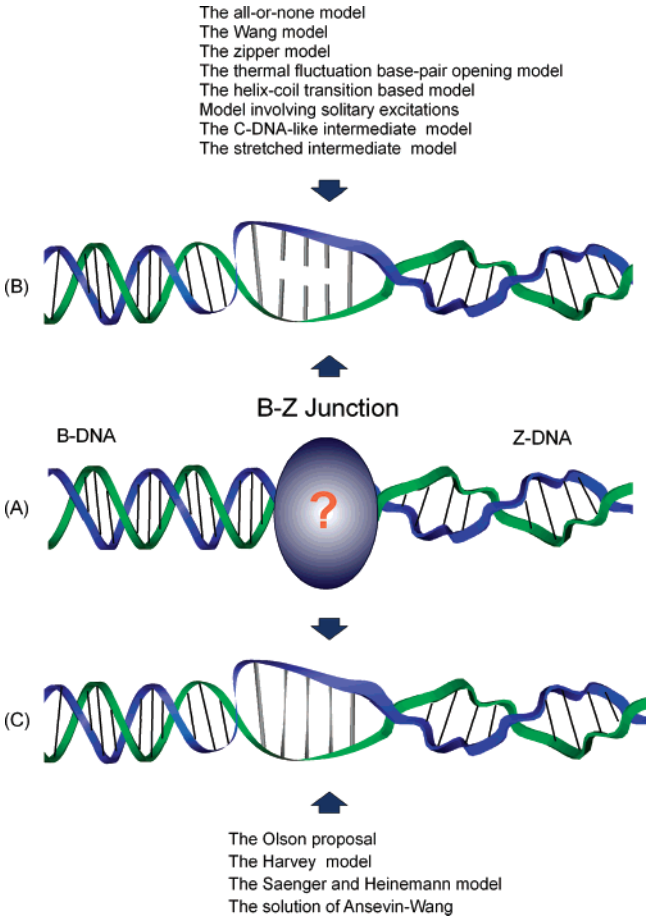


Figure 5. (A) Idealized drawings of a double helix in the B-form (left) and in the Z-form (right). The B−Z junction is represented as before. (B) List of B−Z transition molecular models in which the base pairs of the junction are open. (C) List of B−Z transition molecular models with no disruption of hydrogen bonds. The bars represent the base-pair hydrogen bonds.

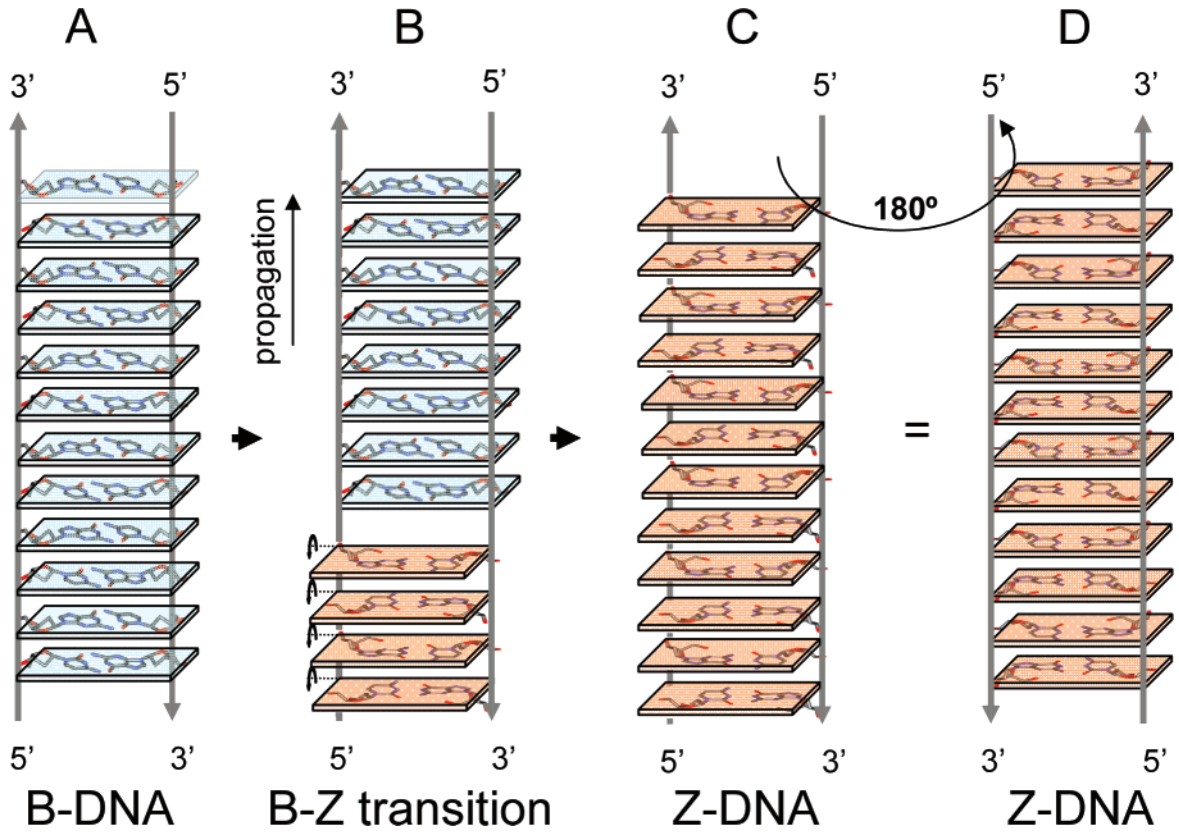


Figure 6. Graphics drawing to illustrate the *chain sense paradox* during the B−Z DNA transition. B-DNA (A) is shown on the left of the panel as viewed from the minor grove side with planes of base pairs projecting away from the observer. Part B shows the progression of the B−Z DNA transition. The gray thick arrows designate the 5′ to 3′ OH progression of standard Watson−Crick backbone chains in B-DNA (A) on the left and counter-Watson−Crick directions in Z-DNA (D) on the right. The identity of the two structures (C and D) on the right of the equilibrium is indicated by an equal sign, showing that a rotation of the base pair plane on the glycosidic bond is equivalent to a reversal of chain directions.

Table 4. Classification of Models Described for the B−Z Transition Discussed in the Review

Models that involve ...				
base-pair opening		base-pair rotation without base-pair breakage		others
without intermediates	with intermediates	without intermediates	with intermediates	
the all-or-none model[3]	C-DNA-like intermediate model[74]	the Olson proposal[98]	the model of Saenger and Heinemann[97]	a unified B−Z transition model[86]
the Wang model[1]	the zipper model[77]	the Harvey model[79]		an empirical salt-threshold model[49]
the thermal fluctuation base-pair-opening model[90]	the stretched intermediate model[85]	the solution of Ansevin−Wang[82]		
a helix−coil transition based model[91]				
model involving solitary excitations[92]				

the stretched intermediate model,[85] that will also be discussed. Currently, the accepted model of the B−Z transition is *the zipper model*.[77] The model involves the high-energy nucleation of a B−Z junction that then propagates through the DNA polymer until the entire B-DNA polymer is transformed into Z-DNA. On the other hand, although a X-ray diffraction pattern of Z-DNA provided a detailed study of the spatial arrangement of the atoms in a structure, it gives little information concerning the thermodynamic and kinetic properties of the B−Z transition. Thus, it is important to take into consideration models that deal with the macroscopic aspects of the problem. In this report, we center our attention on a number of models concerned with macroscopic aspects of the transition, such as, for example, *the unified biophysical model*[86] of the B−Z transition and *the empirical salt-threshold model*.[49] A summary of models of the B−Z transition is included in Table 4.

3.1. Molecular Models That Involve Base-Pair Opening

Different experiments suggest that a number of base pairs open up during the B−Z transition.[87] Some models that involve base-pair opening during the transition have been proposed and will be described hereafter (Figure 5B). The models of the following section include a brief description of the *all-or-none model*, the first model of this family where the base-pair opening is induced by salt; the *Wang model*, which is the most known model in this category; a model in which the transition is facilitated by thermal fluctuation base-

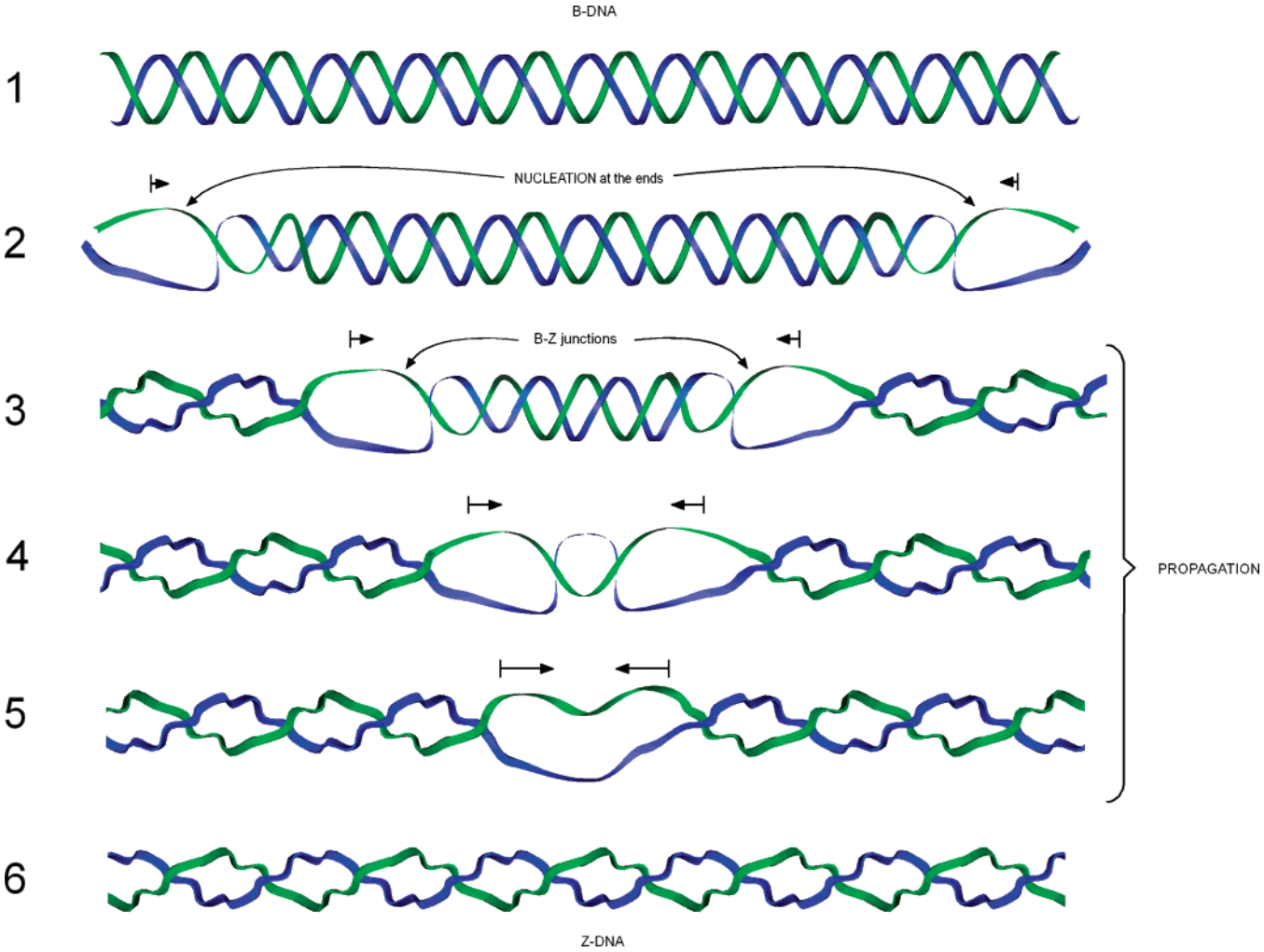


Figure 7. Schematic drawings of the B−Z transition of a poly[d(G−C)·d(G−C)] polymer according to *all-or-none*-like models. The polymer (1) under certain environmental conditions forms nucleation sites only at the ends (2). The propagation reaction moves the junctions in opposite directions in a cooperative way (3−5) until all DNA polymer is in the Z-form (6). The arrows indicate that the motion rate of the junction is proportional to the number of base pairs that are in the Z-form.

pair opening, a model for the B−Z transition of DNA involving solitary excitations; and, finally, the *helix−coil transition based model*, an approach also applied to the helix−coil transition in proteins.

3.1.1. An All-or-None Model

In 1972, to explain experimental results concerning the B−Z transition, a model that connects data obtained by CD with some elementary molecular processes was proposed. It is important to notice that, at this stage, the structural properties of the B- and Z-helical forms were unknown in detail and although the inversion of the circular dichroism spectrum suggested a change of the helix sense, it could not prove it. In any case, Fritz M. Pohl and Thomas M. Jovin anticipated a comprehensive molecular mechanism for the B−Z transition involving the nucleation step of the B−Z transition in which the opening of a number of base pairs at the end of the oligomer takes place. The model would be supported by two conditions: (i) *a steady-state condition*, where the concentration of intermediate states and their change with time were negligible so that only molecules that were entirely in B- or in Z-form would exist in measurable concentration, and (ii) *the nucleation at the end condition*, where the nucleation event in a given oligomer takes place only at the ends of the oligomer (see Figure 7).[3] In this model, the overall equilibrium constant between the B- and Z-forms would be the product of the individual equilibrium constants of every base pair of the chain multiplied by a correction factor. The correction factor (or nucleation factor) would be related to the difficulty of forming the first and second base pairs from a coiled state. As the process is reversible, a nucleation parameter for the B-form and another for the Z-form must be taken into account. The kinetic formulation of the propagation and the nucleation events gives an expression that relates the chain length with the relaxation time for a polymer longer than the junction.

The most important pitfall of the model is related with the conditions of steady state and nucleation at the ends of the polymer. In fact, the assumption that the nucleation event in a given oligomer takes place only at the ends of the molecule does not agree with the experimental data. In fact, there are evidences that suggest that local Z-DNA segments exist in naturally occurring DNA. For example, alkylation of guanine residues in DNA by mitomycin C results in changes found in the high-salt form of the alternating d(G−C)$_n$ polymer.[88] Moreover, CD studies of DNA in high-salt solutions also suggest that segments of the molecule may convert to Z-DNA[89] as an indication that local segments of Z-DNA can be found in the middle of B-DNA when driven by the impetus of guanine alkylation or by high-salt solutions.

The model also lacks prediction capacity for long polymer lengths or when the rate constant for the polymer formation and the rate constant for the base-pair opening have approximately the same value.

On the other hand, the assumption of the steady-state condition is not in agreement with experimental results as reported in the literature,[74] where it has been shown that the B−Z transition induced by salt in a synthetic poly[d(G−C)·d(G−C)] polymer does not follow the steady-state condition[3] but goes through an intermediate, the B*-form. In fact, the B- to Z-DNA transition of poly[d(G−C)·d(G−C)] induced by salt does not behave as a two-state transition as established by the steady-state condition in the *all-or-none model* but goes through an intermediate, the B*-form, and, then, transforms gradually to the Z-form.[74] In this case, the double helix does not dissociate into single strands and transforms from the B*-form to the Z-form point-by-point along the chain with a small amount of open states in which the bases are unpaired.

In any case, the variation of chain length is important for a quantitative description of cooperative phenomena in linear polymers. This problem was considered in detail by Pohl in 1983 using poly[d(G−C)·d(G−C)] ranging in length from 6 to 100 base pairs by applying a variant of the *all-or-none* model.[58] The model predicts increasing B−Z transition rates with decreasing polymer length.

Finally, in 1987 a series of $d(C-G)_n$ oligomers were studied by UV and CD spectroscopy at different temperatures and NaCl concentrations. The analysis of the melting data, assuming an *all-or-none model*, and the kinetics of the B−Z transition support a mechanism by which the Watson−Crick hydrogen bonds were broken before the bases flipped over separately and eventually stacked, re-forming the hydrogen bonds.[75]

3.1.2. The Wang Model

In 1979 a paper described the result obtained when the DNA fragment $d(C-G)_3$ crystallized as a left-handed DNA with Watson−Crick base pairs and an antiparallel organization of the sugar phosphate chains. The paper described the molecular structure of a Z-DNA form at atomic resolution and a molecular model for the B−Z transition.[1] The model described the B−Z transition as involving base-pair opening before rotation, as in the case of the *all-or-none model*, but with accurate information about the Z-DNA structure and notable differences relative to conditions that the model must follow. In contrast with the case of the *all-or-none model*, the nucleation at the ends of the DNA molecule is not a premise for this model. In fact, structural data indicate that the phosphate−phosphate distance across the helix in the Z-DNA form is larger for the d(GpC) segment than for d(CpG). The phosphate distance across the d(GpC) segment resulted to be 15 Å in the Z-form, close to that found in the B-form (17.5 Å). Thus, a B−Z junction could be made at this point but not at the middle of a d(GpC) segment. This suggested that a segment of Z-DNA in the middle of B-DNA probably would involve an even number of nucleotides. However, it is important to note that in Z-DNA, relative to B-DNA, the base pairs are stacked on opposite sides of a line joining the two phosphate groups across the helix. This means that the B−Z junction will have a stacking discontinuity (Figure 8), which might result in a kinking of the molecule. The origin of the *anti-syn* alternation of bases observed in the Z-DNA conformation that produces the characteristic zigzag of the backbone lies in the rotation of the guanine residue about the glycosidic bond, resulting in the *syn* conformation. To remain in an *anti* conformation in the Z-DNA, the cytidine residue, base plus sugar, has to rotate 180°.

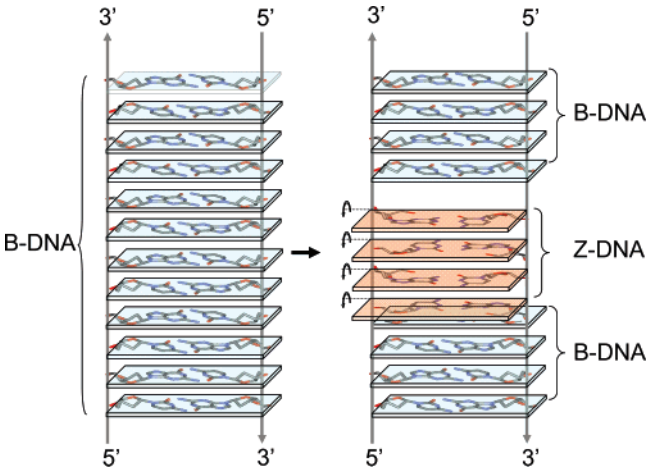


Figure 8. Diagram illustrating the topological changes occurring when a fragment of B-DNA is converted into Z-DNA. The bases which turned upward are indicated by arrows. The rotation is shown by shading in reddish one surface of the bases. Rotation of the guanine base about the glycosidic bonds produces dG in the *syn*-conformation while for dC both cytosine and deoxyribose rotate. The diagrammatic representation also shows that the bases in the Z-DNA segment do not stack as in the B-DNA.

3.1.3. The Thermal Fluctuation Base-Pair-Opening Model

As described, although the B−Z transition is mainly driven by changes in salt concentration, there is a temperature dependence of the transition over a small interval of salt concentration.[65] In the model proposed in 1993 by Chen and Prohofsky, the B−Z transition is facilitated by thermal fluctuation base-pair opening.[90] The transition is characterized by domain formation and domain growth rather than by an instantaneous transition involving all the DNA segments. The model assumes the simultaneous existence of both a B-domain and a Z-domain separated by a junction with disrupted conformation and unpaired bases. This assumption does not fit both *the all-or-non model*, where the nucleation occurred at the ends, and *the Wang model*, where the nucleation occurs at the ends or in a B−Z junction. In the model proposed by Chen and Prohofsky, the temperature and salt concentration dependences of the B−Z transition are calculated using a modified self-consistent phonon approximation theory (better known as MSPA theory).[76] The major modification from the standard theory is the incorporation of a thermal expansion in the determination of the effective force constant.

3.1.4. A Helix–Coil Transition Based Model

To explain increases in B to Z transition rates with increasing polymer length of poly[d(G−m^{5}C)·d(G−m^{5}C)] in the presence of salt,[59] a combinatorial model was proposed for calculating the average length of B- or Z-form tracts at the middle point of the B−Z equilibrium as a function of the chain length. This model is similar to a more formal combinatorial approach applied to the helix−coil transition in proteins.[91] The following conditions are assumed by this model: (i) the source of cooperativity is the unfavorability

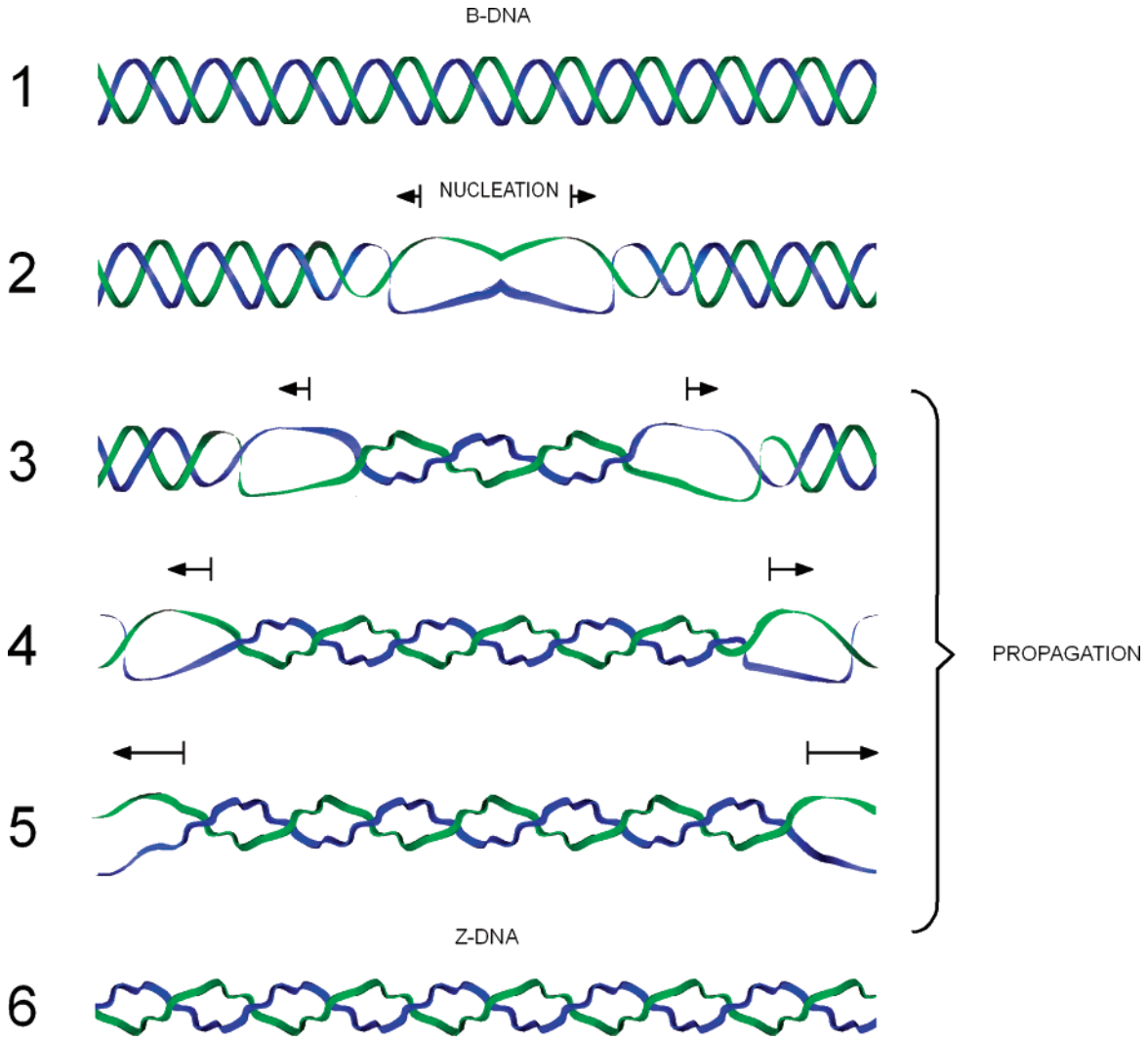


Figure 9. Various steps of the zipper model of the B−Z transition. The polymer in the B-DNA conformation (1) forms a high-energy nucleation step involving two B−Z junctions (2). The propagation of Z-DNA takes place as the junction migrates in opposite directions along the DNA strands in a cooperative way (3−5) until all polymer is in the Z-DNA conformation (6).

of B−Z junctions, (ii) the B−Z transition is two-state and intramolecular, and (iii) the B−Z equilibrium at the midpoint of the transition is represented by an intrinsic equilibrium constant equal to unity for individual base pairs in the absence of cooperativity. This assumption is not made in the model proposed for the helix−coil transition in proteins due to the presence of end effects in the polynucleotide.[59]

The increases in B to Z transition rates with increasing polymer length of poly[d(G−m^{5}C)·d(G−m^{5}C)] in the presence of salt,[59] confirmed lately,[49] are in direct contrast with other experimental data that stated increasing B−Z transition rates with decreasing polymer length.[3,58] An essential difference between this model and the *all-or-none* based models is that the present model favors a rate-limiting internal nucleation event occurring within the polymer tract (see Figure 9), in agreement with *the zipper model*,[77] which predicts increasing B to Z rates with increasing polymer length.

These discrepancies were explained[59] by a nucleation event dominated by end effects for short oligomers but not for long ones. Thus, nucleation in *the all-or-none model* is considered to be occurring predominantly at the ends of oligomers since this requires formation of only a single B−Z interface instead the two associated with internal nucleation within the helical tract. This scheme predicts slower kinetics with increasing polymer length. This behavior is displayed by shorter oligomers in high-salt solutions.[58] However, for both long and short polymers in high-salt solution, experimental data show that the B−Z transition is not totally accomplished.[49] So, nucleation at the ends of the polymer tract is not a sufficient reason to explain these cases of incomplete B−Z transition processes.

3.1.5. Model Involving Solitary Excitations

The model proposed here is very similar to the Wang model[1] except that this case explicitly requires the flipping-over processes to occur at the outside of the helix. Thus, in this model[92] the hydrogen bonds of a base pair in the B-DNA conformation are first broken. Then, each of the separated bases of a pair can rotate out of the helix around an axis parallel to the helical axis. As suggested previously,[93] those open states may be described as solitary excitations, which were discussed using a simplified two-dimensional plane base-rotator model.[94,95] The model predicts the existence of the Hoogsteen base pairs which would provide the first evidence for the hydrogen bond breaking mechanism in the B−Z transition process.

3.2. Molecular Models That Involve Base-Pair Rotation without Base-Pair Breakage

At this point, the basic question that could be formulated is: does double-stranded DNA possess sufficient conformational flexibility to permit the flipping of a single base pair without breaking the Watson−Crick hydrogen bonds? Undoubtedly, changes in backbone torsional angles will allow considerable extension of the DNA helix, since intercalation of a variety of molecules is a well-established experimental fact.[96] It has been demonstrated from structural measurements that there is considerable and sufficient conformational flexibility in the DNA to carry out the B−Z transition.[73] A detailed analysis of the topological differences between B-DNA and Z-DNA led researchers to propose other possible molecular mechanisms for the B−Z transition. Here, we will account for models that involve base-pair rotation without base-pair breakage (Figure 5C). A brief description of the *Olson model*, the first model described of this type, the *Harvey model*, the most known model in this category, and the solution of Ansevin−Wang,[82] that seeks to replace the Z-DNA model proposed earlier, will be mentioned. We will end up with a third view where the B−Z transition is carried out through an intermediate conformation.[97]

3.2.1. The Olson Proposal

One of the first molecular mechanisms for the B−Z transition of this category was proposed in 1983 by Olson.[98] In that model, two base pairs flip simultaneously, going from a face-to-face to a back-to-back orientation by passing through a transition in which they are side-by-side. The base-pair hydrogen bonds are kept intact, and the energetic cost of distorting bonds and bond angles is kept down by the simultaneous variation of two or more parallel nonadjacent torsional angles in the sugar phosphate backbone. The more important complications of the model are related with both the cooperativity and the topology of the B−Z transition.[79] Thus, as occurs with the base-pair-opening based models, no simple explanation of cooperativity is provided by this model, and regarding the topology of the B−Z transition, the molecular mechanism proposed requires that while a guanosine is going through the *anti* state, its neighbor must go through the *syn* state. This movement would produce a wrong sense in the half twist at the cytidine deoxyribose. This movement cannot be topologically correct.

3.2.2. The Harvey Model

In 1983 Stephen C. Harvey proposed a molecular mechanism for the B−Z transition, which tried to resolve the problems faced by the *Olson model*. So, in the *Harvey model* the base pair rotates in unison without base-pair breakage and unwinding of the helix.[79] According to this model, the activation process takes place in two phases and the most energetic price is paid when the cavity between the B and the Z segments is open. Then, the base pair flips and the cavity moves down to a new position. The process is facilitated by longitudinal DNA breathing modes. This transition model was proposed subjected to the following constraints: (i) all Watson−Crick hydrogen bonds were kept intact; (ii) all non bonded contact distances were always larger that 3 Å; (iii) sugar puckers were kept within the classical range for double-helical nucleic acids;[73] and (iv) although the separation of the terminal two bases varied from 7 to 14 Å and they rotated to vary the helical twist angle,

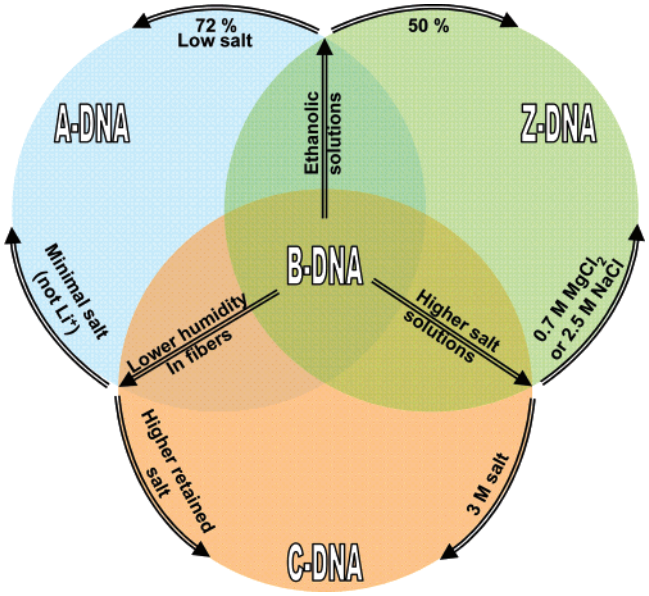


Figure 10. Classical scheme showing families of naturally occurring DNAs[146] as possible intermediates during the B−Z transition process. Transitions between DNA families are induced by changing ionic strength or solvent polarity in solution or changing salt content in fibers or films and the relative humidity. Critical salt or ethanol concentrations[3,47,147] give midpoints rather than endpoints of transitions. (Reproduced with permission from ref 146 (http://www.nature.com). Copyright 1980 Nature Publishing Group.)

they were kept parallel and their lateral sliding displacements were never greater than 3 Å. Harvey stated that even with the restriction imposed by these constraints, it would be possible to carry out the transition.

There were two advantages to the mechanism proposed in this model over that described by Olson.[98] First, it accounted for the cooperativity of the B−Z transition in a simple way because the author postulated that it is relatively costly from an energetic point of view to form the cavity and relatively economical to flip each base pair within it so that the cavity would remain open when moving down the helix as the transition is propagated. Second, this theoretical representation provided a correct topology for the rotation of every base pair. The model might be in agreement with NMR experimental data, which seem to suggest that hydrogen bonds are intact during the B−Z transition.[79]

3.2.3. The Solution of Ansevin–Wang

Concerned with the observation that the right−left transition of a double helix is too easy when no change occurs in the direction of the backbone progressions,[83,99] Ansevin and Wang suggested in 1990 that the answer to the steric dilemma known as *the chain sense paradox* could be found if the transition would proceed from a B-like to a Z-like helix that would retain the conventional Watson−Crick backbone directions. Such a helix was named Z[WC]-DNA to indicate that the helix has a zigzag backbone and chains that possess the orientation chosen by Watson−Crick for B-DNA.[82] The structural solution for the Z[WC] helix (Figure 11) was elaborated under certain constraints: (i) It had to be left-handed, (ii) it had to have 12 base pairs per turn, (iii) it had to have a pitch of 44 Å, (iv) it had to possess a dinucleotide repeat, and (v) it had to have Watson−Crick chain directions. When compared with the *Wang model*,[1] in both representations a portion of the major face can be seen near the center

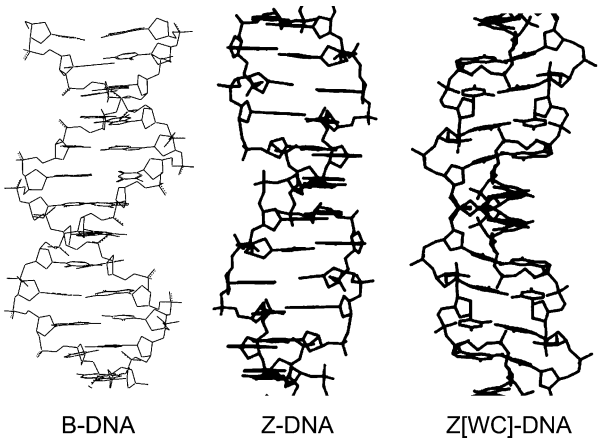


Figure 11. Comparison of B-DNA with two left-handed DNA models, the Z-DNA[1] and the Z[WC]-DNA[82] models (in bold).

while segments of the spiraling single groove are exhibited at the bottom and the top. The main differences between them are that the guanine-N2 amino group of Z[WC]-DNA forms an additional hydrogen bond to an oxygen of the 5′-adjacent phosphate group that supports the left-handed helix. Moreover, the base stacking patterns are different, with the distinction in the backbone directions being the most important difference.

3.3. Molecular Model That Involves Intermediates with Disruption of Hydrogen Bonds

The existence of intermediate states in the B−Z transition has resulted to be one of the more fruitful predictions to understand the transition at the molecular level.[74,82,85,97] To recall the conditions in which naturally occurring DNA structures can be obtained and the relationship between them as possible intermediates in the B−Z transition process, we refer the reader to the classical drawing of Figure 10.

As seen before, the equilibrium approach assumes that intermediates in the transition never accumulate and can be neglected. Noncooperative approaches recognize intermediates in the transition but ignore the fact that the B−Z transition is cooperative. The compromise was *the zipper model*,[77] the more currently accepted model of the B−Z transition. Another scheme that at the molecular level involves an intermediate during the B−Z transition process postulated the disruption of hydrogen bonds during the transition. This scheme was one of the first proposals made about the existence of intermediates in the B−Z transition process.[74] Finally, *the stretched intermediate model* postulates that the B−Z transition occurs through an intermediate S-type DNA.[100]

3.3.1. The Zipper Model

In 1983 Lawrence J. Peck and James C. Wang, studying topoisomers of plasmids containing $d(C-G)_n \cdot d(C-G)_n$ inserts, were able to show that the B−Z transition within the alternating C−G was induced by negative supercoiling, with the transition being highly cooperative. The free energy for the transition was evaluated from a statistical mechanical study of the data today known as *the zipper model*.[77] This formulation gives a simple molecular description of cooperative transitions between different conformations within a biopolymer. The model divides the transition into two phases, initiation and propagation (see Figure 9). Thus, *the zipper model* defines a high-energy nucleation step to initiate the formation of the less stable conformation, followed by a number of lower-energy steps for extending this structure throughout the sequence, and had been used with success to analyze the transition from B-DNA to single-stranded DNA,[101,102] cruciforms,[103] triple-stranded DNA,[104] and Z-DNA.[77,105,106]

The structure of the B−Z junction was described in theory as a set of unpaired bases because the B−Z transition requires a junction having an infinite helical twist.[106] Experimentally, each B−Z junction appeared as four base pairs that were sensitive to single-strand-specific nucleases[107] and chemical reagents.[108,109] Figure 9 shows different steps for the B−Z transition in *the zipper model*. The high-energy initial nucleation step involves formation of two B−Z junctions within the $d(C-G)_n \cdot d(C-G)_n$ insert equivalent to eight unpaired bases. The Z-DNA propagates as the junctions migrate in opposite directions along the chain until the entire B-DNA polymer is transformed into Z-DNA. Unfortunately, this model does not reveal many structural and dynamic details of the B−Z transition itself by limiting its application to the thermodynamics of the B−Z transition. In any case, there are very good agreements with experimental results; particularly notable is the successful mach of both the steepness of the transition and the location of the midpoint as the length of the polymer is varied. Some authors support the *zipper model* as the currently accepted picture of the B−Z transition.[85]

3.3.2. C-DNA-like Intermediate Model

On the basis of experimental data obtained by time-resolved CD, Sachio Goto postulated in 1984 the existence of an intermediate during the salt-induced B−Z transition of poly[d(G−C)·d(G−C)] with disruption of hydrogen bonds. This intermediate was named B*-DNA.[74] The existence of that structure was confirmed later when the NaCl-induced B−Z transition of a 16 base pair deoxyoligonucleotide adopted a hybrid form containing both B- and Z-DNA joined by a B−Z junction. This structure has properties of a partially dehydrated intermediate consistent with the behavior of that documented by Goto.[53]

The B−B* transition proceeded nearly instantaneously, and then the B*-form was transformed gradually to the Z-form. Relative to the case of B-DNA, the UV and CD spectra of the B*-conformation were nearly the same and fairly different, respectively. The CD spectrum of the B*-form resembled that obtained for DNA in high-salt solutions and was similar to that of the C-DNA form (see Figure 10). Goto suggested that the B*-form was a rate-determining step in the B*−Z transition and supported the idea that the polynucleotide immediately folded into Z-DNA. As the process was reversible, in the Z−B transition the polymer would not convert directly from the Z-form to the B-form, but through a B*-like form. He suggested, moreover, that the double helix does not dissociate into single strands but transforms from the B*-form to the Z-form point-by-point along the chain in a soliton-like manner with a small amount of open states in which the bases are unpaired.

3.3.3. The Stretched Intermediate Model

Early in 2005 Lim and Feng suggested, by applying the stochastic difference equation to simulate the B−Z transition, that a stretched intermediate could appear as a natural consequence of the unwinding of a DNA oligomer during the structural change.[85] Unwinding tends to destabilize base

stacking interactions and thus results in unhindered stretching of the oligomer that would adopt an intermediate conformation named S-DNA (or stretched intermediate-DNA).[100] The S-DNA conformation is also used to describe overstretched DNA formed during the B−S DNA transition in force-induced overstretching experiments.[110,111] The formation of a stretched intermediate state reduces the backbone energy, thereby increasing the amount of energy available for DNA bases and sugars to overcome the torsional barriers and compensating the energy required for conformational changes. During the B−Z transition, the stretching of DNA has certain advantages. For example, a reduction in the backbone energy also allows the bases to make large amplitude motions by the increase in rise, which removes the strict constraints imposed on rise-dependent orientation variables and, therefore, reduces steric clashes between bases.[112]

The model does not pose any steric dilemma and shows that the chain sense reversal progresses spontaneously. Although the model may have several advantages over other models, it can be applied only to an oligomer because in much longer DNA strands the model has an important shortcoming, that is, the increase in base stacking energy that is needed to be overcome before DNA starts to stretch.[113]

3.4. Molecular Models That Involve Intermediates with No Disruption of Hydrogen Bonds

Although in theory poly[d(G−C)·d(G−C)] polymers have sufficient conformational flexibility to carry out the B−Z transition process without base-pair breakage, a problem remains unsolved with the models described before. They involve a winding of 180° rotation of bases and reorganization of the backbone that create a steric dilemma.[82] To resolve this dilemma, one approach considers that the transition occurs through an intermediate A-type DNA (see Figure 10).[97]

3.4.1. Structural Model of Saenger and Heinemann

The model was proposed in 1989 and postulates that the A-DNA form of poly[d(G−C)·d(G−C)] is metastable and that the B−Z transition induced by salt is really a B ↔ A ↔ Z-DNA transition.[97] The authors suggested that the B−Z transition described as a two state process[24] is but an illusion created by the fact that the A-forms are usually not detected during the B−Z transition. However, an A-form of poly-[d(G−C)·d(G−C)] was observed by CD spectroscopy when TFE was added to an aqueous solution of polymer (Figure 10).[114] In these conditions, an A−Z DNA transition was monitored.[115] According to this model, the reason for the B−Z transition of poly[d(G−C)·d(G−C)] lies in the stacking properties of the d(CpG) steps when they are in the A-form. The ±*gauche* → *trans* conformational change of the α and γ torsion angles of 5′-CpG-3′ opens the structure of guanosine so that the *anti-syn* rotation of the sugar can easily occur, leading to a more stable conformation in which the open A-DNA is changed to the more compact Z-DNA. The right to left transition can occur smoothly, without base-pair opening once the B- to A-DNA conformational change is induced.

3.5. Other Models for the B–Z Transition

The effects of salt on nucleic acids have always been analyzed in terms of a counterion condensation theory, which predicts that fixed concentrations of counterion are found near the DNA, independent of bulk salt concentration. Variations of the ionic distribution near the DNA surface play an important role in determining salt effects on the conformational changes observed. The counterion condensation theory, however, cannot explain the experimentally observed salt effect on the B−Z transition.[116] The dependence of the binding of a variety of ligands to DNA shows that it is not the entropic release of counterions that makes the major contribution to the transition, but rather the changes in the interactions of DNA with its ion atmosphere, which strongly depend on bulk ion concentration.[117] Several polyelectrolyte theories have been used to calculate the interactions of nucleic acids with ions.[118−120] These studies have revealed that the association of ions to nucleic acids is commonly electrostatic and that specific coordination is absent, so that it is not an important aspect of nucleic acid structure.

To differentiate them from other models, it is important to emphasize, at this point, that both *the unified biophysical model* and *the empirical salt-threshold model* make no explicit mention of the intramolecular motion of the B−Z transition.

3.5.1. A Unified Biophysical Model of the B–Z Transition

The purpose of this model[86] was to illustrate that the effects of a number of ions on the B−Z transition of poly[d(G−C)·d(G−C)] and poly[d(G−m^{5}C)·d(G−m^{5}C)] may be understood in the Poisson−Boltzmann model of strongly charged polyelectrolytes in terms of the ionic distribution in mixed salt solutions,[121] without invoking any specific interactions. Thus, one must consider the absolute value of the total free energy difference, rather than its sole variations as a function of the salt.

Theoretically, the DNA molecule is modeled by cylinders with a single uniform charge layer at the surface. The major difference between B-DNA and Z-DNA with regard to electrostatic properties is that the phosphates stick out in the B-form, so that they are well surrounded with solvent whereas in the Z-form they are close to the rest of the DNA. Thus, B-DNA is represented by a hollow cylinder with solvent both inside and outside of the charged surface, and Z-DNA, by a solid cylinder with solvent on the outside only.

When experimental values for the free energy difference are included in this model, it is found that the difference between nonelectrostatic contributions to the free energy differences of poly[d(G−C)·d(G−C)] and poly[d(G−m^{5}C)·d(G−m^{5}C)] provides an explanation about the ionic concentrations used for the B−Z transition induced by salt for these polymers.[9] Moreover, the model also accounts for other properties related with the competition between counterions of different valences, such as the observed invariance of the composition of the counterion sheath at the transition[122] or the disproportionate effect of fractional methylation. In summary, the model shows how a low concentration of multivalent ions can influence the structure of nucleic acids even in the absence of any specific affinity site.

3.5.2. An Empirical Salt-Threshold Model

An empirical expression was deduced from experimental data to explain some largely unrecognized characteristics of the B−Z transition induced by salt and temperature in sized poly[d(G−C)·d(G−C)] and poly[d(G−m^{5}C)·d(G−m^{5}C)] polymers and to predict new ones. The expression relates the degree of the B−Z transition with parameters such as

the counterion concentration present in the solution, the type of salt, the temperature, or the polymer length.[49]

The main achievement of this model is that envisages the existence of a salt threshold that could suggest that the molecular mechanism of the B−Z transition could be a mixture of several of the proposed models. So, the contrasting rates found for the B−Z transition of sized poly[d(G−C)·d(G−C)] and poly[d(G−m^{5}C)·d(G−m^{5}C)] polymers in the presence of salt would have a natural explanation. In agreement with experimental data, up to the salt threshold the model establishes increasing B−Z transition rates with decreasing the polymer length,[58] whereas above the salt threshold the model establishes increasing B−Z transition rates with increasing the polymer length.[49,59] Moreover, the model also agrees with experimental data showing that cooperativity is independent of temperature.[49] This model predicts a length for the B−Z junction of 4−6 base-pairs for poly[d(G−C)·d(G−C)] according to data obtained by Raman spectroscopy and NMR[50,51] but the model also predicts that the length of the junction could be longer for poly[d(G−m^{5}C)·d(G−m^{5}C)] (10−12 base pairs).[49] Finally, an interesting consequence of the model is that in the absence of counterions the temperature is unable to drive by itself the B−Z transition process[31,49] leading to the melting of the DNA molecule.

4. Critical Comments about the Proposed Models for the B–Z Transition

Accepted experimental methods that confirm the existence of a Z-DNA helix do not provide evidence of many of the features of the structure or of fundamental aspects of Z-DNA models. Thus, an examination of Z-DNA models discloses that a greater part of the experimental measurements are centered on only two characteristics of the DNA structure: the dinucleotide repeat and the handedness of the helix. Moreover, if more demanding standards are applied, a number of disagreements are revealed between observations and the theoretical outlook provided by Z-DNA models, namely, (i) the reversed chain direction relative to B-DNA,[82] (ii) the increase/decrease of the B−Z transition rate with the polymer length,[58,59] (iii) the existence or not of the Z-DNA conformation at low ionic strength and, therefore, the existence of a double transition like the Z ↔ B ↔ Z transition as a function of the counterion concentration,[60−62] and (iv) the impossibility to drive the Z- to B-DNA transition by temperature in the absence of counterions.[63]

The main conclusion is that serious problems are encountered when it is assumed that any currently recognized structure serves as a wholly satisfactory model for polymeric or naturally occurring Z-DNA because any molecular model of the B−Z transition must be able to explain the key experiments. So, the assumption that more than one model must be considered at the same time to explain the reported aspects of the B−Z transition process is justified by itself when the data available from the literature are put together.

The idea that the B−Z transition has intermediate states[74,97] proved to be very productive as time passed by. For instance, the problem of the reverse chain direction relative to the case of B-DNA had to be posed to eliminate the steric dilemma of the B−Z transition. Looking for differences with crystallized Z-DNA,[1] the proposed Z[WC]-DNA[82] circumvented certain discrepancies observed between the two structures assuming that the Z-DNA helix observed by crystallography was not the Z-DNA helix with its chain sense reversed, but an alternative Z-DNA version whose chain sense was the same as that of Watson−Crick B-DNA. The possible existence of such an intermediate of the B−Z transition facilitated the explanation of several criticisms described before.[82] For example, the existence of the intermediate state introduces the possibility that bulky adducts such as AAF or others that react with poly[d(G−C)·d(G−C)] facilitate or even force the B−Z transition to be carried out by virtue of its zigzag distorting effect on a regular B-DNA.[123] This observation has been confirmed by experiments.[124,125] However, the existence of an intermediate could provide an alternative explanation. If the major groove binder is an α-helical peptide,[33,45,126] an intermediate stretching transition is produced[126] and now the B−Z transition is facilitated by the intermediate species. However, it would be interesting to point out that the existence of intermediates in the B−Z transition of synthetic poly[d(G−C)·d(G−C)] polymers could place a constraint upon attempts to infer a structural model of the transition only by molecular modeling methods because the intermediate dictates the way in which the transition process should proceed.

On the other hand, it is not clear from experimental data whether the nucleation event in a given oligomer takes place at the ends,[3] in a B−Z junction,[40,48] or within the polymer tract.[1] In any case, independently of its location within the polymer tract (see Figure 12), the nucleation event is energetically expensive to form the nucleation site to flip each base pair. In contrast, it would be relatively economical to flip the rest of the base pairs as the transition is propagated cooperatively.[79,127] In fact, after the unfavorable positive free energy contribution in the nucleation process is overcome, the free energy needed for the additional steps becomes negative and the transition progresses spontaneously. Such a mechanism is similar to practically all B−Z transition models proposed up to date.[77,79,97,127]

At this stage, the concept of cooperativity (a parameter considered in several models) arises in a natural way to explain contrasting experimental observations found in the literature, such as, for example, the increasing[49,59]/decreasing[49,58] B−Z transition rates when the polymer length increases. Thus, a B−Z transition is considered to be a cooperative process if the probability to flip each base pair depends on the number of base pairs which have flipped before. Such a definition points to the polymer length as the basic parameter related with the cooperativity of the process[3,49,58,59] and consequently with the rate of the B−Z transition (see text of Figure 12).[49,58] Therefore, for poly[d(G−C)·d(G−C)] and at fixed temperature the rate of the B−Z transition would decrease with increasing polymer length[58] up to an ionic strength threshold[31] and would increase with increasing polymer length if the ionic strength is higher than the threshold.[31,49] Then, the location of such an ionic strength threshold depends on both the temperature and the type of counterion used.[29] The existence of the salt threshold raises a problem relative to what happens with DNA at low and high ionic strengths. It is well-known that the two DNA strands in a duplex are held together by hydrogen bonding and by base stacking of the paired bases. The base-pair opening in double-stranded DNA is inhibited not only by the length of the polymer but also by the counterion concentration of the medium and by the GC/AT ratio of the DNA sequence.[127−131] So, at high ionic strength the base-pair opening is quite difficult, especially for long poly[d(G−C)·d(G−C)] polymers. Consequently, for short

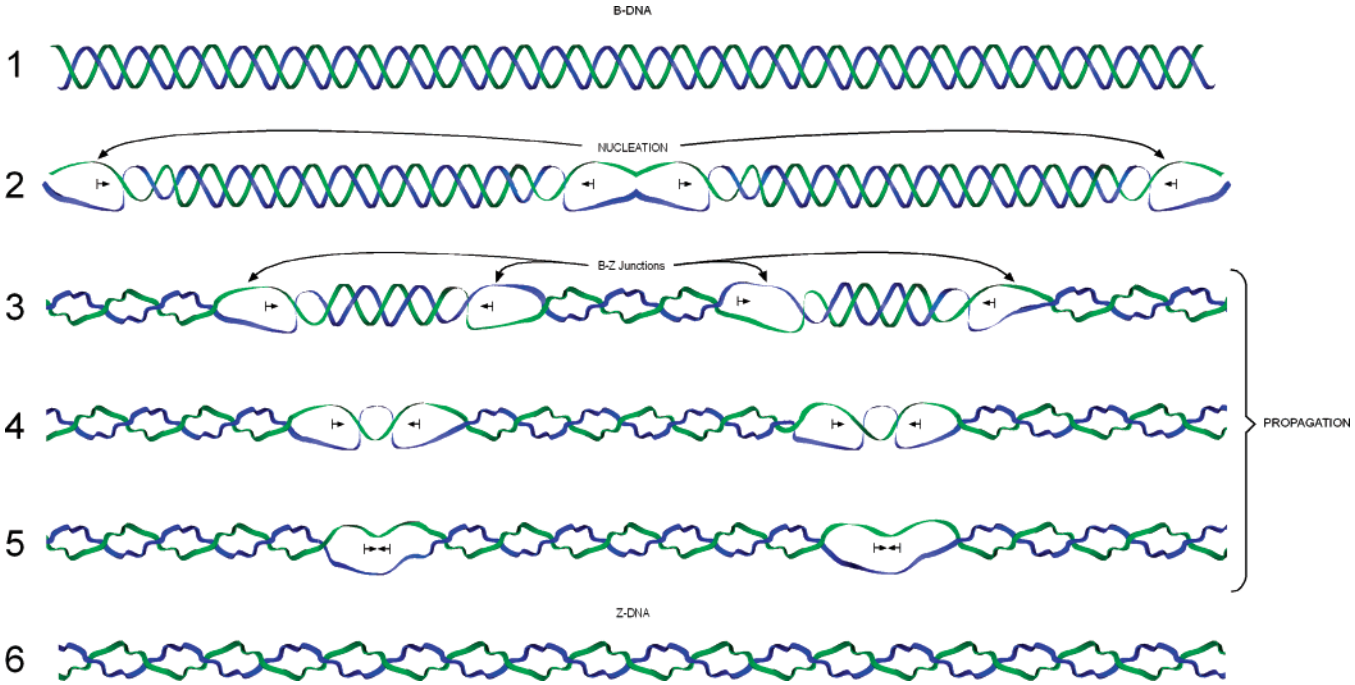


Figure 12. Schematic drawings of the B−Z transition of a poly[d(G−C)·d(G−C)] polymer according to a mixture of models that could explain some experimental observations. The polymer (1) under certain environmental conditions forms nucleation sites within the polymer tract and also at the ends (2). The propagation reaction moves the junctions in opposite directions in a cooperative way (3−5) until all DNA polymer is in the Z-form (6). The arrows indicate the motion rate of the junction, which is proportional to the number of base pairs that are in the Z-form.

polymers the base-pair opening is easier at low ionic strengths because the hydrophobic forces involved in base stacking are minimized. If we consider that the stacking energies are influenced in poly[d(G−C)·d(G−C)] by both composition and sequence and that the stacking energies are lower for the stacking patterns GpC and CpG sequences,[132] the base-pair opening within the poly[d(G−C)·d(G−C)] tract would be favored in such conditions. Experimental data also indicate that the destabilizing effect on the DNA double helix of decreasing the salt concentration causes a decrease in the overstretching force.[133,134] It should be pointed out that, as described above, the empirical salt-threshold model accounts for some experimental data which would be in agreement with base-pair-opening models. However, it could also be applied to models which do not imply base-pair opening. As has been indicated above, at low salt concentration the more stable form of the synthetic double-stranded polymer poly(dG-m^{5}dC)·poly(dG-m^{5}dC) seems to be the Z-DNA conformation, in contrast with the cases of classical experiments described before. Thus, the existence of a double transition like the Z ↔ B ↔ Z transition as a function of the counterion concentration in poly(dG-m^{5}dC)·poly(dG-m^{5}dC) has been described.[60] Such an observation has also been reported in the absence of any oligovalent cation in the solution.[61] An experiment was designed to explain these contrasting results, and the data obtained indicated that at low counterion concentration a Z-DNA form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) appears to be stabilized by trace amounts of divalent cations (Mg^{2+}, Ca^{2+}, etc.) present in the sample. Thus, only when such cations had been removed and monovalent sodium ions were present at low concentration would the polymer adopt the classical B-DNA conformation.[62]

One important prediction of the *empirical salt-threshold model* is that by varying the environmental conditions (i.e., counterion concentration, type of salt, temperature, etc.) and the type of polynucleotide it might be possible to obtain either B ↔ Z[49,65] or Z ↔ B[64,65] transitions induced by temperature. So, the model predicts a possible sequence of Z ↔ B ↔ Z ↔ B ↔ ... transitions generated through various combinations of the environmental conditions of the polymer.[49] However, in all cases the presence of salt in the polynucleotide solution is a *sine quanon* condition to carry out the conformational B ↔ Z or Z ↔ B transitions. Then, in the absence of counterions and with increasing temperature, the B ↔ Z or Z ↔ B structural transitions are forbidden[29,31,49] and only a melting transition is observed in the DNA.[63]

The concept of a B−Z junction is particularly useful *in the zipper model* where a high-energy nucleation step involves first the formation of the B−Z junction (6−8 base pairs) followed, then, by the propagation of the junctions in opposite directions along the DNA chain while the original site is converted to Z-DNA (Figure 9). This scheme fits well with the proposal of the possible existence of several nucleation points in a DNA molecule due to the stochastic election of the nucleation site when the nucleation energy is higher than that necessary to form a nucleation spot. Unfortunately, however, the zipper model does not reveal many structural and dynamic details of the B−Z transition itself by limiting its application to the thermodynamics of the B−Z transition.

Molecular dynamic simulation has been successful in computational studies of biological macromolecules[135,136] in spite of the fact that the most important limitation of the technique is its restriction to short time scales. However, the boundary value formulation[137] of functionals and actions is useful if it is necessary to know the evolution of one state to another without having to specify exactly the initial or environmental conditions. However, the problem that must be solved is that the discrete version of the action cannot be used with larger time scales. To solve such a problem, an approach based on stochastic modeling of numerical errors introduced by a finite difference formula[138] was proposed

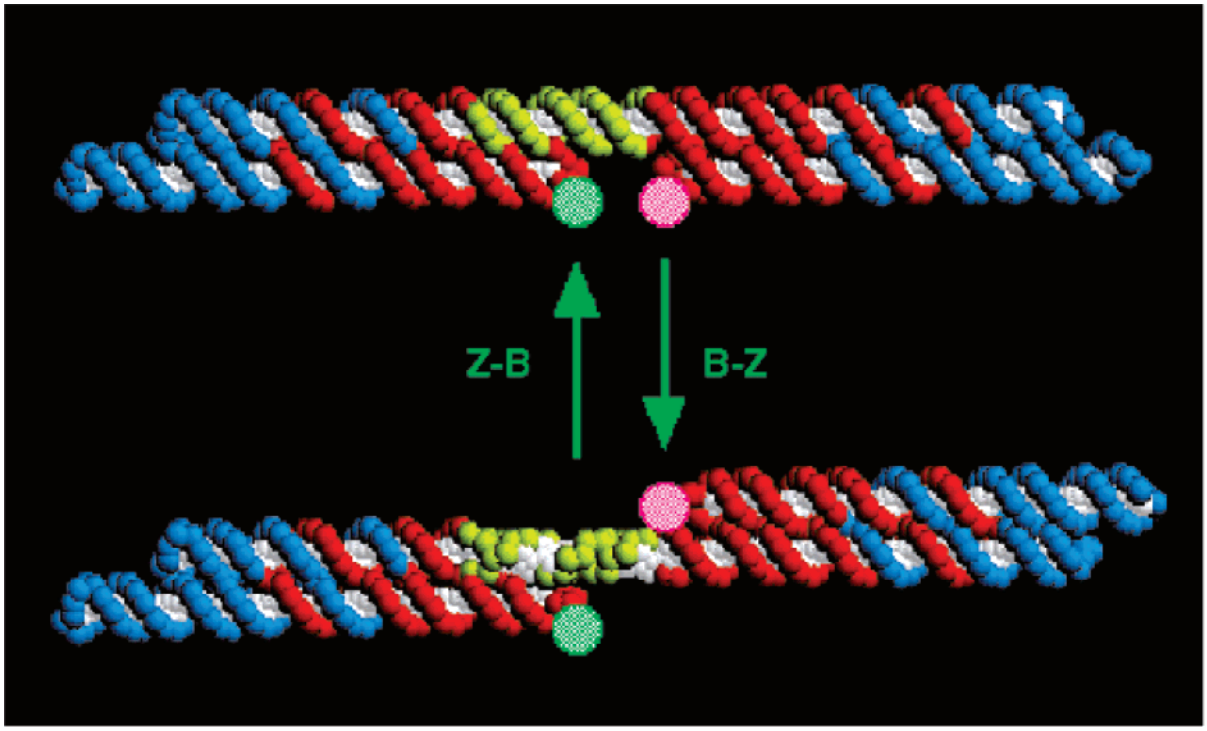


Figure 13. Design of a nanomechanical switch based on the B−Z transition. Top, molecular model of the molecule constructed entirely from right-handed B-DNA. Each nucleotide is shown as two spheres, a colored one for the backbone and a white one for the base. Three cyclic strands are shown, one in the center drawn as a red strand with a central yellow segment, and two blue strands on the ends that are each triply catenated to the red strand. Fluorescent dyes are drawn schematically as stippled green (Fluorescein) and magenta (Cy3) circles attached to the free hairpins near the middle of the molecule. At the center of the connecting helix there is a 20-nucleotide region of proto-Z DNA in the B-DNA conformation, shown in yellow. When the B−Z transition takes place, this yellow portion becomes left-handed Z-DNA (bottom). When the transition occurs, the two DX molecules change their relative positions, increasing the separation of the dyes. It is possible to cycle this system in both directions.[148] (Reproduced with permission from ref 148 (http://www.nature.com). Copyright 1999 Nature Publishing Group.)

to model the B−Z transition process. Under these conditions, the simulation was carried out in the *stretched intermediate model*.[85]

5. Concluding Remarks and Future Trends

In the past few years, significant progress has been made toward the understanding of the molecular mechanism of the B−Z transition in [d(G−C)·d(G−C)] polymers. The mechanisms proposed and herein reviewed tried to shed light on the conformational change underlying the common characteristics that several reported models share. Thus, theoretical and experimental data pointed toward the existence of B−Z transition intermediates that clarify a great number of criticisms arising from most of the current models and open new research trends for the future. The formation of a nucleation site in the DNA and its energy-cost appear as common characteristics from various models and lead to the useful concept of cooperativity and to postulate the existence of a salt threshold capable to transform contrasting results into complementary.

The Z-DNA structure does not exist in nature as a stable conformation but as a transient structure occasionally induced by biological activity. Today, the scientific community is paying attention to some aspects of Z-DNA as the discovery of certain classes of proteins that bind to it with high affinity and specificity[23] or the evidence that shows B−Z-conformational changes in the hippocampus of Alzheimer's brain[139] progresses. It is known that Z-DNA-binding proteins can act as potent effectors of gene expression *in vivo*,[140] participating also in the pathology of poxviruses.[23] Another actual field and future trend of research, the so-called DNA and RNA nanodevices, represents a toolbox for controlling structural states of nucleic acids objects. As a particular case, there are several B−Z transition based nanodevices used as on−off switches that consist of two DX molecules linked by a shaft of double-stranded DNA (see Figure 13).[141] These nanodevices enable the control of the transition in space, by sequence requirements, fixing how much of the DNA will undergo the B−Z transition. The need for Z-DNA promoting conditions could also allow the modulation of the transition with time.[49] The value of nanodevices will be addressed when a multidisciplinary community establishes goals for their use. At this point, it is worth mentioning that advances are also necessary in the improvement of nucleic acid force fields such as the BMS nucleic acid force field that produce environment and sequence dependent DNA conformations that closely mimic experimentally derived structures[142] and also are used for molecular dynamic simulations, as the accuracy of computational or theoretical studies depends on the force field used.

In our view, future research efforts will have to concentrate on further analysis of the following issues: (i) the biological role of the Z-DNA structure, (ii) the manipulation of Z-DNA by tweezers, (iii) advances in computational biology in the Z-DNA field, (iv) the B−Z transition based nanomachines, and (v) the use of the B−Z transition as a research tool to study drug−DNA interactions. The latter aspect can be regarded as a prerequisite for the elucidation of the molecular basis of drug−DNA interactions[143] and its correlation with the cytotoxic effects observed.[144]

6. Glossary and Abbreviations[168]

AAF	2-(acetylamino)fluorene ($C_{15}H_{13}NO$). Used in the study of liver enzymes and the carcinogenicity and mutagenicity of aromatic amines as a positive control and also as a research tool.
ADAR1	the editing enzyme double-stranded RNA adenosine deaminase, which converts adenine to inosine in pre-mRNA. The enzyme has an N-terminal domain that binds tightly to Z-DNA.
CD	circular dichroism. A method that measures differences in absorption of right- and left-circularly polarized light as it passes through a sample solution. CD replaced ORD in most applications, since the resulting spectra are easier to interpret and more simple to compare.
A-DNA	a DNA structure that is more compact than B-DNA and found only in a dehydrated state
B-DNA	the standard right-handed double-helical structure of DNA
B*-DNA	an intermediate C-DNA-like form proposed to exist during the B−Z transition
C-DNA	a DNA structure obtained at high Na^+ content and humidity conditions intermediate between those required to produce A- and B-DNA
S-DNA	a stretched intermediate DNA form proposed to exist during the B−Z and B−S DNA transitions
Z-DNA	left-handed conformation of DNA that can be formed from conventional DNA under adequate sequence and environmental conditions
Z[WC]-DNA	Z-DNA-like form whose chain sense is the same as that of Watson−Crick B-DNA
DX	supramolecular device consisting of two rigid DNA "double-crossover" molecules connected by double-helical turns
IR	infrared
NMR	nuclear magnetic resonance. NMR spectroscopy is used to study the chemical structure of molecules. The technique replaces X-ray crystallography for the determination of protein structure.
ORD	optical rotatory dispersion. ORD measures the effect in which optically active samples rotate the plane of linearly polarized light.
pRW751	a derivative of pBR322 plasmid containing $d(C-G)_{13}$ and $d(C-G)_{16}$ segments
Z-RNA	a left-handed form of RNA double helices. The Z-RNA structure shows several conformational features significantly different from those of Z-DNA.
TFE	2,2,2-trifluoroethanol ($C_2H_3F_3O$). TFE has been widely used as a structure inducing cosolvent, and it is assumed to stabilize helical structures in native proteins and peptides.
UV	ultraviolet

7. Acknowledgments

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