

# Implications and Concepts of Polyamine-Nucleic Acid Interactions

Burt G. Feuerstein, Loren D. Williams, Hirak S. Basu, and Laurence J. Marton

Brain Tumor Research Center, Department of Neurological Surgery, School of Medicine (B.G.F., H.S.B., L.J.M.), Department of Laboratory Medicine (B.G.F., L.J.M.), and Department of Pediatrics (B.G.F.), University of California, San Francisco, California 94143 and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (L.D.W.)

**Abstract** Modeling, x-ray diffraction, and solution studies have contributed to the understanding of interactions between polyamines and nucleic acids. Polyamines stabilize a variety of unusual DNA structures and conformations *in vitro*, including both the left-handed Z and the right-handed A DNA. In addition, polyamines condense DNA and may be important in bending specific sequences. Investigations into the mechanisms of these effects provide support for both specific and nonspecific interactions between polyamines and DNA. Although exact relationships between the binding of polyamines and conformational changes in nucleic acids are still being clarified, polyamines remain important candidates for regulators of DNA conformation *in vivo*.

**Key words:** nucleic acid conformation, DNA, DNA conformation, polyamines, spermine, spermidine, DNA bending, A DNA, B DNA, Z DNA, anthracyclines, DNA/ligand interactions, polyamine function

During the past 10 years, broad advances have been made in the understanding of interactions between polyamines and DNA. This field has received attention because polyamine concentrations vary by orders of magnitude as a function of cellular growth state and are important regulators of cell growth and differentiation. The interactions of polyamines with DNA are a natural starting point for explorations of specific functions for polyamines in living cells. Theoretical predictions as well as studies of polyamine-nucleic acid systems in crystals and in solution support the idea that polyamines induce a variety of conformational changes in DNA. Although the mechanisms, specificity, and biology of these changes are still being elucidated, the evidence indicates that they are biologically important. Here we review selected studies focusing on specificity in the binding of polyamines to DNA and on the resulting conformational changes.

## MODELING STUDIES

Early models of polyamine-nucleic acid complexes were based on direct electrostatic interactions between the positively charged amino groups on polyamines and the negatively charged phosphates on DNA [1]. Zhurkin [2] explained the stabilization of A DNA by polyamines by postulating that the amino groups of polyamines and phosphate groups of DNA interact more favorably in this configuration than in B DNA.

Feuerstein et al. [3,4] used molecular mechanics [5] to evaluate the interactions of spermine with B DNA in both alternating purine/pyrimidine and homopolymeric sequences. Four spermine docking positions were studied: within the minor groove, along the phosphate backbone, bridging the minor groove, and within the major groove (Fig. 1). This method models a collection of atoms by calculating their energy and then optimizes their positions by finding an energy minimum. These calculations predict that spermine interacts best in the major groove of alternating purine/pyrimidine sequences. Spermine forms hydrogen bonds with N7s and O6s of purines, with O4s of pyrimidines, and with phosphate oxygens, and hydrophobic interactions oc-

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Address reprint requests to Dr. Burt G. Feuerstein, Department of Neurological Surgery [Box 0926], c/o The Editorial Office, 1360 Ninth Avenue, Suite 210, San Francisco, CA 94122.

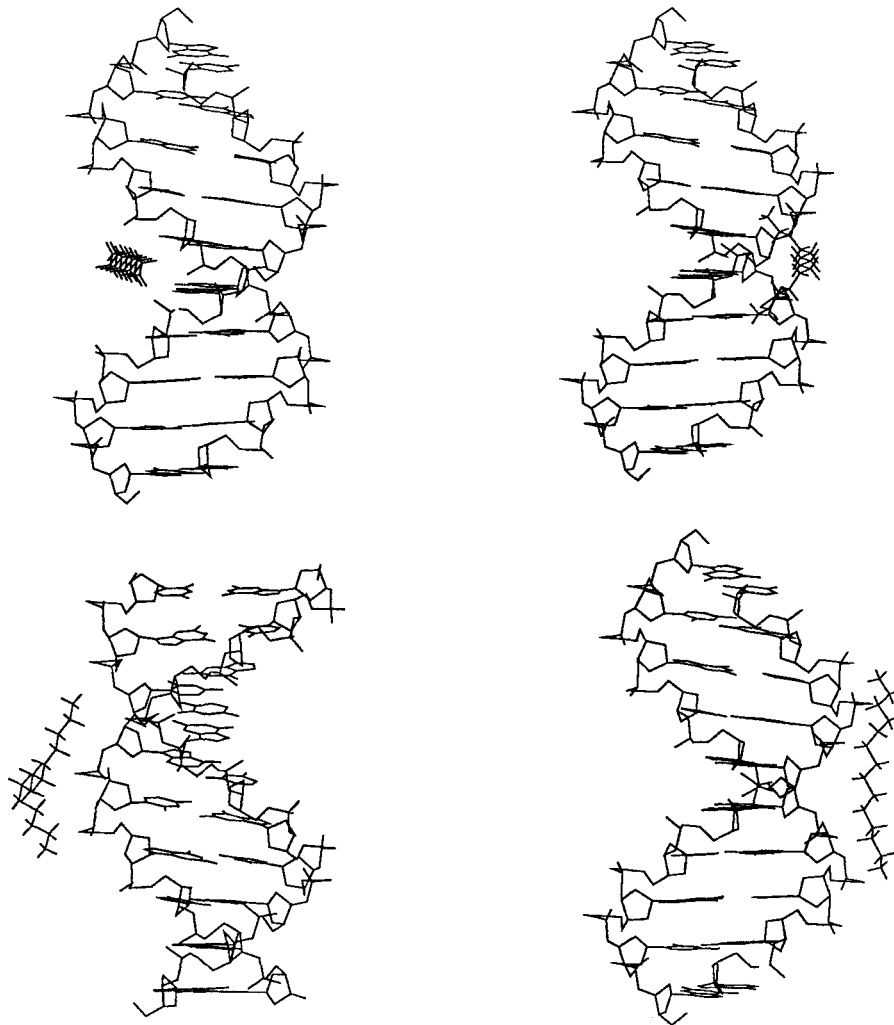


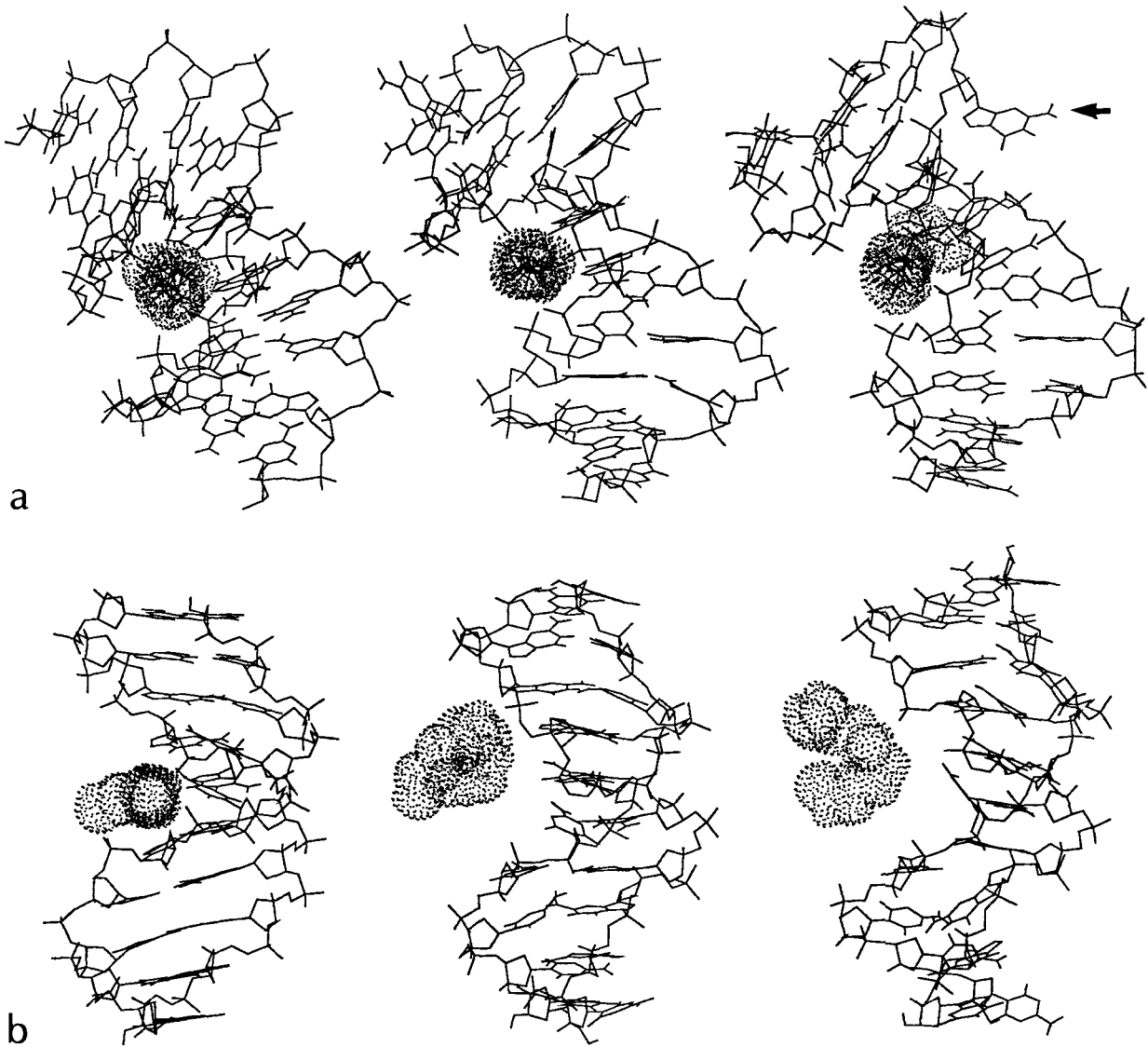
Fig. 1. Four different docking positions for spermine on B DNA: within the major groove (upper left), within the minor groove (upper right), along the phosphate backbone (lower left), and bridging the minor groove (lower right). The upper structures and lower right structure show the major groove on the left and the minor groove on the right.

cur between the aliphatic carbons of spermine and the methyl group of thymine. These interactions cause DNA to bend, narrowing the major groove and widening the minor groove (Fig. 2a). Bending also causes changes in sugar pucker that result in shorter interphosphate distances.

Like alternating purine/pyrimidine sequences, homopolymeric sequences bind spermine best in their major grooves, but this interaction does not result in substantial bending (Fig. 2b). In both alternating and homopolymeric DNA sequences, the binding of spermine to the minor groove and to the phosphate backbone is less favorable than its binding to the major groove. In homopolymeric sequences, however, the differences between the strengths of binding to the major groove and to the minor groove are

smaller. In general, modeling studies predict that the specific sequences that bind spermine most tightly are those that undergo the greatest conformational changes.

Molecular dynamics [5] were also used to model interactions between spermine and both unbent homopolymeric and bent alternating purine/pyrimidine sequences [6]. This method uses Newtonian mechanics to calculate trajectories for each atom after heat has been added to the system, allowing simulation of molecular interactions over time. These studies confirm that spermine bound to bent DNA is more stable than spermine bound to unbent DNA (Fig. 2). Spermine remains associated with the major groove of bent alternating purine/pyrimidine sequences throughout 40 picoseconds of simula-



**Fig. 2.** Energy minimization and molecular dynamics models of the spermine-DNA interaction. **a:** Spermine in the major groove of alternating guanine/cytosine polymers  $d(GC)_5, d(GC)_5$ . Note that the dimensions of the major groove are smaller than those in Figures 1 and 2b and that the DNA is bent. **Left:** the spermine/DNA complex after energy minimization and before molecular dynamics. **Middle:** the complex after 20 picoseconds of dynamics. **Right:** the complex after 40 picoseconds of dynamics. Note that spermine remains within the major groove and that after 40 picoseconds one guanine base has rotated out of its normal stacking arrangement (arrow). **b:** Spermine in the major groove of homopolymers  $d(G)_{10}, d(C)_{10}$ . The dimensions of the major groove are similar to those in Figure 1, and the timing of molecular dynamics is the same as in Figure 2a. Note that spermine does not remain in the major groove.

tion, but spermine associated with unbent homopolymeric sequences quickly escapes into the solvent. In addition, the interaction of spermine with bent DNA causes enough destacking to rotate one dG on its glycosyl bond out of the normal base stacking configuration (Fig. 2a, right).

The narrower major groove, altered sugar puckering, and reduced interphosphate distances seen in B DNA models after the binding

of spermine are characteristic of A DNA. The changed groove size and interphosphate distances are also characteristic of Z DNA, which has a deep and narrow groove and short interphosphate distances. The rotation of dG out of the normal configuration also suggests Z DNA, in which purine residues are altered from anti to syn. This evidence that spermine favors structures similar both to A DNA and to Z DNA corresponds with spermine's known ability to

stabilize both of these structures over B DNA (see the following sections).

The prediction that spermine induces a sequence-specific bend in DNA may have important implications in the regulation of genomic tertiary structure. Because the tertiary structure of DNA influences transcription, the involvement of polyamines in tertiary structure indicates their possible involvement in gene expression. Spermine-induced bending may be involved in the packaging of viral DNA [7]. Similarly, polyamines may affect the packaging of eukaryotic DNA in supercoils from nucleosomes to chromosomes.

### CRYSTALLOGRAPHIC STUDIES

The information obtainable from an x-ray crystal structure is largely defined by the degree of order in the crystal. The orderliness of the crystal determines both the resolution of diffraction data and, ultimately, the degree of detail in the Fourier electron density maps used to build and refine molecular models. Crystals of nucleic acids complexed with spermine have a wide range of crystalline order, in some cases limiting the amount of information they provide. Thus, crystals formed by the spermine-Z DNA complex diffract to 1 Å, allowing detailed atomic maps to be drawn, whereas those of spermine and yeast-tRNA<sup>Phe</sup> diffract to 2.7 Å, indicating a relatively large degree of disorder.

The x-ray structure of spermine alone in phosphate buffer shows that the amino groups of spermine form direct hydrogen bonds to phosphate oxygens [8]. Complexes of spermine and nucleic acids, however, may not be this simple. In the following sections we review studies of nucleic acid-spermine crystals, focusing on the specificity of interactions and the resulting conformational changes.

#### Yeast-tRNA<sup>Phe</sup>

Yeast-tRNA<sup>Phe</sup> requires the addition of spermine to produce crystals that diffract to adequate resolution [9,10]. The yeast-tRNA<sup>Phe</sup> crystal of the greatest resolution (2.7 Å) contains two bound spermine molecules and four hydrated magnesium ions [11]. One spermine molecule is located in the deep groove, extending from one end of the D stem into the anticodon stem. Although detailed descriptions of intermolecular interactions remain ambiguous at this resolution, three of the amino groups of this spermine molecule appear to be near enough to

phosphate oxygens of the yeast-tRNA<sup>Phe</sup> to form hydrogen bonds with them. This spermine molecule appears to narrow the groove by approximately 3 Å and to contribute to a 25-degree bend in the helical axis. (Note the similarity between this description and that of the B DNA model in the preceding section). The second spermine binds to the minor groove of the D stem near its intersection with the acceptor stem. This spermine molecule also appears to be near enough to form several hydrogen bonds with phosphate and ribose oxygens. However, the relative importance of specific interactions, such as hydrogen bonds and hydrophobic contacts, cannot be definitively established at this resolution.

### A DNA

The structure of spermine bound to the major groove of an A DNA octamer, a duplex of d(GTG-TACAC), has been determined to 2.0 Å resolution [12]. The amino groups of the spermine molecule, bound to the floor of the deep groove on a crystallographic two-fold axis, form hydrogen bonds to bases but not to phosphate or ribose oxygens. Each terminal amino group forms a hydrogen bond with the O4 of a thymine, and each central amino group forms a hydrogen bond with the N7 of a guanine. Spermine also forms extensive hydrophobic contacts with the DNA, the most important of which appear to involve the central methylenes of spermine and the 5-methyl group of a thymine. The interaction of spermine with bases in this structure provides a model for the binding of spermine to RNA and to DNA-RNA hybrids, known to favor the A conformation. This interaction may also help us to understand the regulatory properties of polyamines in gene expression, because regulatory proteins may bind through functional groups and hydrophobic interactions in the major groove.

### Anthracycline-DNA Complexes

Anthracyclines are a family of widely used chemotherapeutic agents that intercalate into DNA. The activity of the anthracyclines is extremely sensitive to modification [13]. Daunomycin, for example, is effective in the treatment of acute leukemia. Adriamycin, which differs from daunomycin only by the addition of a hydroxyl group at the 14 position (Fig. 3), is more effective in the treatment of solid tumors. A newer analogue, 4'-epiadriamycin, differs from Adria-

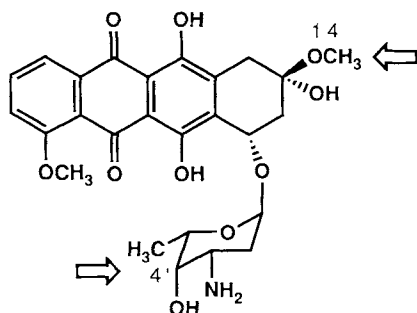


Fig. 3. Daunomycin. The sites at which the structure is modified for the formation of Adriamycin and 4'-epidriamycin are indicated by arrows.

mycin only by a stereochemical inversion at the 4' position.

Each of these anthracyclines has been cocrystallized with the DNA hexamer d(CGATCG) and solved to approximately 1.5 Å resolution [14,15]. Thus, in this series of cocrystals, it is possible to probe the effects of small chemical differences on spermine's interaction with DNA and on the resulting conformational changes. In each cocrystal, an anthracycline molecule intercalates at each of the two d(CG) steps of the hexamer duplex, and two spermine molecules bind symmetrically in the major groove (Fig. 4). The conformation of the DNA is nearly the same in the three cocrystals, and the general characteristics of its interactions with spermine remain consistent. Each spermine molecule lies in the major groove and forms hydrophobic contacts with both the DNA and the anthracycline. At least one methylene group per spermine molecule contacts a 5-methyl of thymine and a hydrophobic region of the anthracycline, preventing water molecules from contacting the hydrophobic regions of the three compounds. Comparison of these complexes with anthracycline-DNA complexes crystallized in the absence of spermine indicates that five to seven water molecules are displaced from hydrophobic groups by the binding of a single spermine molecule [16].

Despite these similarities, the conformation of the bound spermine is different in the three crystals. The only covalent differences among the constituent molecules of the three complexes involve hydroxyl and O-methyl groups of the anthracyclines located in the minor groove and remote from spermine. Through a reorganization of the solvent, however, these groups are the most likely cause of the differences observed in the conformation of the spermine molecules. Whether such solvent-mediated effects on sperm-

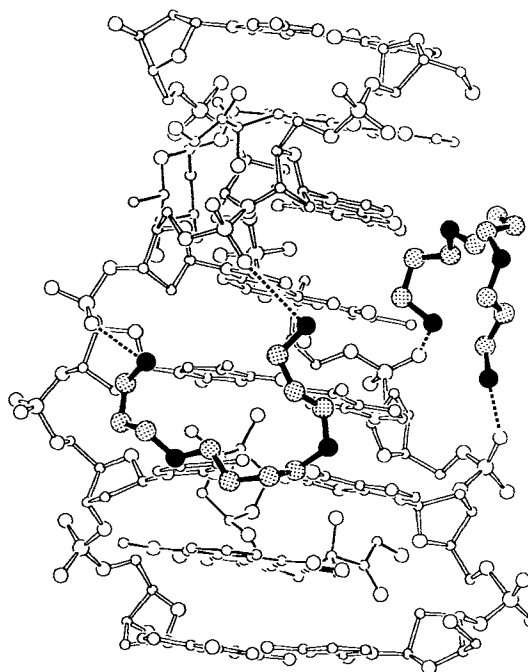


Fig. 4. Spermine molecules bound in the major groove of the d(CGATCG)-4'-epidriamycin complex. The DNA is drawn with hollow bonds, the anthracycline with thin solid bonds, and the spermine with thick solid bonds. The hydrogen bonds linking the spermine with the DNA are drawn with dashed lines. The nitrogen atoms of spermine are black, and the carbon atoms are stippled. A twofold axis in the center of the DNA duplex relates the two spermine molecules to each other. (Reproduced from Williams et al. [15] with permission of Oxford University Press.)

ine conformation are related to the biological functioning and clinical properties of anthracyclines remains unanswered.

## Z DNA

The self-complementary DNA hexamer d(CGCGCG) crystallizes in the left-handed Z conformation in the presence of magnesium and in the presence of spermine [17,18]. Both of these crystals diffract to better than 1 Å resolution, allowing detailed examination of the molecular structure and of the positions of solvent, cations, and polycations. The magnesium and spermine complexes of Z DNA assume almost identical conformations.

The complex of Z DNA and spermine contains two crystallographically distinct spermine molecules. One of these binds to Z DNA primarily by forming direct hydrogen bonds to the convex surfaces of two different (but symmetry-related) Z DNA duplexes. These hydrogen bonds are to the O6 and N7 positions of guanines, three of

which are on one DNA duplex and a fourth of which is on an adjacent duplex. The second spermine molecule in the Z DNA crystal interacts simultaneously with three different Z DNA duplexes. This spermine, however, forms fewer hydrogen bonds to DNA bases than does the first, and it forms more bonds to phosphate oxygens. Like those bound to yeast-tRNA<sup>Phe</sup>, the spermine molecules are located in a region where negatively charged phosphate oxygens lie close to each other. Viewing the nucleic acid down its

vertical axis, spermine molecules appear to form a cage, effectively neutralizing the negative charge and allowing adjacent helices in the lattice to approach one another closely (Fig. 5).

Calculations indicate that the floors of DNA grooves, where spermine binds in yeast-tRNA<sup>Phe</sup>, A DNA, and DNA-anthracycline complexes, are regions of high electronegative potential [19,20]. Charge-charge interactions, therefore, in addition to hydrogen bonds, hydrophobic interac-

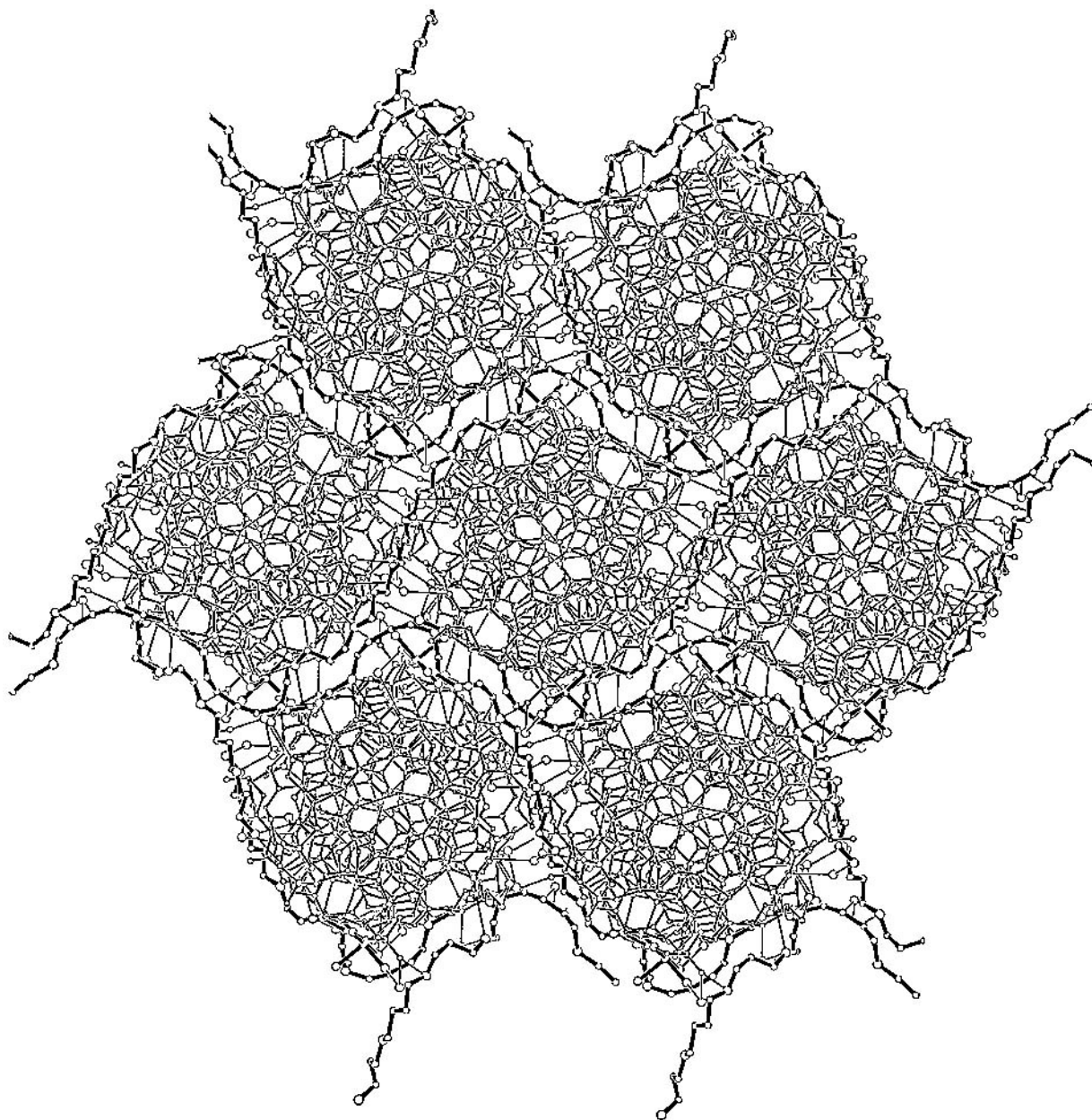


Fig. 5. View down the helical axis of a crystallized Z DNA-spermine complex. The DNA is drawn with thin lines, and the spermine molecules are drawn with thick lines.

tions, and van der Waals contacts, appear to make important contributions to the stabilities of these complexes. Favorable charge-charge interactions do not require hydrogen bonds between the amino groups of spermine and the phosphate oxygen of a nucleic acid. In the case of Z DNA, in which spermine molecules are located in regions where phosphates form close intermolecular and intramolecular contacts, it is clear that a single spermine molecule can neutralize either the charges of two phosphates on a single DNA molecule or those of phosphates on adjacent DNA molecules. Lattice packing, therefore, allows interaction of DNA duplexes with each other. Because recombination also involves interaction between DNA duplexes, it is possible that it is regulated by polyamines.

### SOLUTION STUDIES

It is well established that DNA can assume various conformations depending on base sequence, ionic strength, and other environmental factors [21]. These include the classical right-handed B DNA [22] and A DNA [23], left-handed Z DNA [17,24], and the triple helix [25,26]. The B/Z transition may be important to gene expression [27] and to phasing of nucleosomes during chromatin condensation [28], whereas the triple helix may be involved in recombination and repair processes [29]. DNA bending is sequence-dependent and occurs both in the presence and in the absence of DNA binding proteins [30]. The ability of low concentrations of spermidine or spermine to induce the B/Z transition [31,32], to induce bends in DNA

[33,34], to stabilize A DNA, and to stabilize the triple helix in both nonenzymatic (Richard Shafer, personal communication) and enzymatic [35] systems demonstrates the possible importance of polyamines to DNA conformation *in vivo*.

Early studies of interactions between polyamines and DNA in solution emphasized the ability of polyamines to condense and aggregate DNA [36]. Because other polycations such as  $\text{Co}(\text{NH}_3)_6^{3+}$  cause similar effects, Bloomfield and Wilson [37] proposed that interactions between polyamines and DNA obey Manning's counterion condensation theory [38]. This model describes DNA as a linear distribution of negative charge and polyamines as delocalized point charges. Porschke's study of viral DNA condensation supports this model: the binding kinetics of polyamines are too fast to allow for specific site binding [39]. A nuclear magnetic resonance (NMR) study of a spermine-dodecanucleotide mixture [40] also suggests lack of specificity in spermine-DNA interactions. However, because NMR signals represent an average of signals from all ligand molecules in solution, signals from specifically bound spermine molecules might be masked by spermine molecules interacting nonspecifically with DNA.

Other experimental data, in fact, suggest specificity in polyamine-DNA interactions (Table I). Spermine inhibits the binding of an antibody directed toward both the phosphate backbone and the convex surface of Z DNA, enhances the binding of an antibody directed toward the convex surface alone, and does not affect the binding of one directed toward the phosphate back-

TABLE I. Sequence-Specificity in Spermine-DNA Interactions

Reference number	Subject	Method	Observations
37	DNA condensation	Flow linear dichroism	Specific toroidal shaped condensate
33	DNA condensation	Electron microscopy	pH dependence of size of toroids
47, 58	Ligand-DNA interaction	Enzyme assay	Differential effect on activity of different enzymes
59	DNA structure	Circular dichroism, NMR	Affecting structural change at 1 spm:40 BP
42	Anti-Z antibody/Z-DNA interaction	Radio-immunoassay	Differential effect on antibody binding
60	Spermine-DNA interaction	Gel filtration	Higher affinity for GC-rich DNA
35	Spermine-DNA interaction	Electric birefringence	Decreasing relaxation time for (dA-dT) and increasing relaxation time for (dG-dC)
34, 43	Spermine-DNA interaction	H-D exchange kinetics	Increasing iminoproton exchange rate for (dA-dT) and decreasing rate for (dG-dC)

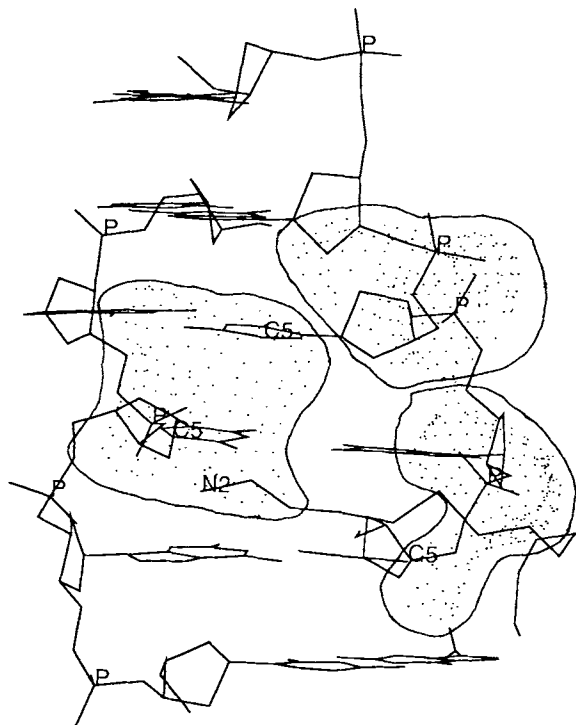


Fig. 6. Binding sites of spermine on Z DNA. A Z DNA crystal is shown with three putative antibody binding sites represented by dots. One site (left) is marked by two C5 atoms. The binding of spermine enhances the binding of this antibody. The surface surrounding both the lower phosphate moiety and C5 (lower right) is a putative site for a second antibody, the binding of which is inhibited by spermine. The surface surrounding the remaining phosphates (upper right), a third antibody binding site separated from the convex surface, is unaffected by spermine.

bone alone (Fig. 6) [41]. Thus, spermine appears to have a specific binding site on the surface of Z DNA in solution similar to one of its two binding sites identified on a Z DNA crystal. A second site observed in the crystal structure appears to bridge three adjacent DNA molecules and may

result from DNA packing in the crystal lattice; therefore, it may not be seen in solution.

Stopped flow hydrogen-deuterium exchange studies support the existence of a polyamine-induced bend in B DNA, as predicted by the energy minimization studies discussed previously. Spermine increases the imino proton exchange rate of poly d(AT) · poly d(AT) and decreases that of poly d(GC) · poly d(GC), but it does not appreciably change the exchange rates of the amino protons in either polynucleotide [33]. A similar increase in the imino proton exchange rates of poly d(AT) · poly d(AT) and poly d(A) · poly d(T) in the presence of spermidine has also been observed through NMR relaxation kinetic studies [42]. These observations can be explained by a spermine-induced bend in DNA. In poly d(GC) · poly d(GC), the imino proton is protected from solvent exchange by hydrogen bonds in both the major and minor grooves (Fig. 7, right). Polyamines, by increasing the melting temperature, stabilize the double strandedness of the DNA, which in turn decreases the rate of imino proton exchange. Poly d(AT) · poly d(AT), however, has no hydrogen bond in the minor groove. Widening the minor groove of this polynucleotide should then increase the accessibility of imino protons to solvent, resulting in an increased rate of imino proton exchange (Fig. 7, left).

Further evidence for polyamine-induced bending of DNA comes from a recent study comparing the effects of spermidine and Integration Host Factor (IHF), a protein known to bend DNA, on the binding of gpNU1, a subunit of lambda terminase, to lambda DNA [43]. Apparently, spermidine and IHF can substitute for each other in allowing the protein to bind. It is possible that each produces a bend or other

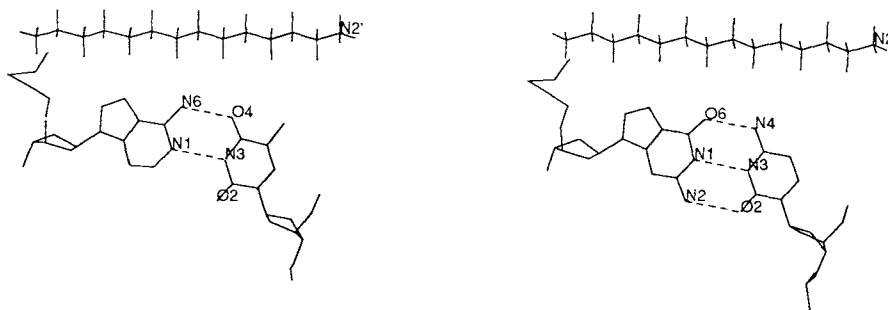


Fig. 7. Spermine in the major groove of an AT base pair (left) and a GC base pair (right). The imino proton is between the N1 of the purine and the N3 of the pyrimidine. Amino protons are between O6 and N4 and between N2 and O2 on GC, and between O4 and N6 on AT. If the base pairs bend toward spermine, the minor groove opens, exposing the imino proton of the AT base pair to solvent. In the case of GC, the hydrogen bond between N2 and O2 protects the imino proton from solvent.



conformational change in DNA necessary for the binding of gpNU1.

Electric birefringence studies of spermine complexed with poly d(GC) · poly d(GC) and poly d(AT) · poly d(AT) provide strong evidence for a spermine-induced bend in poly d(AT) · poly d(AT) [34]. The stiffening seen in GC-containing polymers under low-salt conditions is consistent with the B/Z transition that takes place in poly d(GC) · poly d(GC) [44; Feuerstein BG, unpublished data].

Although no direct evidence of polyamine-induced DNA bending has been reported, the preceding data imply that spermine and spermidine induce specific conformational changes in B-form poly d(GC) · poly d(GC) and poly d(AT) · poly d(AT) and that specific binding sites exist on the surface of Z DNA in solution. A sequence-specific binding site for spermine is also indicated by preliminary results on the effects of spermine on the fluorescence polarization of ethidium-DNA complexes: the conformational changes induced by spermine in the heteropolymer poly d(AT) · poly d(AT) appear to differ from those induced in the homopolymer poly d(A) · poly d(T) [45]. The enzymatic digestion of spermidine-DNA aggregates also indicates that the binding of the polyamine to DNA is specific [46]. Because the activity of condensed DNA as a substrate is a function of polyamine structure, spermine-DNA interactions cannot be completely explained by Manning's theory [38]. Plum and Bloomfield [47] recently reported differences of two orders of magnitude in the binding constants of three trivalent cations (spermidine, N<sup>8</sup>Me-spermidine, and Co(NH<sub>3</sub>)<sub>6</sub>) with poly(dA-dT) under identical reaction conditions. The specificity of these interactions between polyamines and DNA may be explained by taking into account both the cation radii [48] and the residual dipole moments of different DNA base sequences [49]. In addition to these determinants, the specific conformational changes described earlier may also contribute to sequence-specific interactions between polyamines and DNA.

#### CONCLUSIONS AND FUTURE DIRECTIONS

Polyamines are excellent candidates for regulators of the secondary and tertiary structures of nucleic acids. Not only do polyamines promote B/Z and B/A transitions and stabilize triple helices, but data from modeling, crystals, and solutions indicate that they also stabilize or

cause bends in nucleic acids. These conformational changes may have both general and specific effects on nucleic acid functions, affecting biological processes such as chromatin condensation, nucleosome phasing, transcriptional regulation, and DNA recombination. In addition, they may affect the cytotoxicity of such DNA-directed agents as anthracyclines or alkylating agents [50,51]. It will now be important to investigate how DNA conformation and related biologic processes are affected by intracellular polyamine concentrations. Levels of intracellular polyamines can be manipulated either with specific inhibitors of the biosynthetic enzymes that synthesize them or with mutants selected for defective biosynthesis. Previous data have favored the existence of polyamine-induced conformational changes in cultured cells [52].

Evidence is still only circumstantial regarding the important question of specificity in polyamine-DNA interactions. On one hand, polyamines can be localized to specific positions in a crystal lattice, and modeling studies imply that stable interactions may be possible. Solution studies, on the other hand, show that polyamines can also interact nonspecifically with nucleic acids. The difficulty is in locating the sites of specific interactions in a sea of nonspecific ones. One possible approach might be to isolate monoclonal antibodies for specific oligonucleotide-polyamine complexes.

The interest in polyamine-nucleic acid interactions has been driven by a search for definition of the biologic functions of polyamines. Polyamine depletion inhibits cell growth, at times without cytotoxicity, and influences gene expression [53]. Recently, polyamine analogs with greater affinity for DNA than their parent compounds but with poorer ability to aggregate it have been found to inhibit growth. These analogs may compete with natural polyamines for binding sites on DNA [54,55,56], ultimately leading to growth inhibition. To test hypotheses such as these, detailed investigations of their effects on living cells and on nucleic acid sequences derived from these cells are necessary. The polynucleotides or small oligonucleotides often used in physical and theoretical studies may not have the secondary or tertiary structure important to a specific gene. After the sequences regulated by polyamines in vivo are described, the ready availability of cloned sequences should enable physical investigation of

these specific fragments and their interactions with polyamines.

Investigation of chromatin and nuclei may also provide insight into how polyamines affect structural details of specific genes in a more natural setting. Recent reports demonstrate that the sensitivity of cell nuclei to micrococcal nuclease, DNase II, and DNase I is dependent on polyamine concentration [57; Basu HS, unpublished data]. Thus, it is likely that polyamines regulate chromatin conformation. Further study of specific genes will allow researchers to determine whether these alterations are structurally associated with changes in the regulation of transcription or synthesis.

There is significant interest in studies of polyamine-nucleic acid interactions. Polyamines regulate nucleic acid conformation *in vitro* and may have a similar role *in vivo*. Further progress in this field will depend on productive interactions between cell biologists, biochemists, and physical and theoretical chemists.

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