Base Only Binding of Spermine in the Deep Groove of the A-DNA Octamer d(GTGTACAC)[†,‡]

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ABSTRACT: The crystal structure of a complex of spermine with the DNA octamer d(GTGTACAC) has been determined at 2.0-Å resolution. The alternating sequence adopts an A-DNA conformation with a novel purine–purine extra-Watson–Crick hydrogen bond involving the central guanine G3 (G11) and adenine A13 (A5) in the deep groove. The oligocation spermine binds in the floor of the deep groove by interacting with the bases and assumes an S-shape. Its dyad is coincident with that of the DNA, reminiscent of repressor binding to B-DNA. The terminal and central ammonium groups of the top half of spermine form hydrogen-bonding interactions to the 5′-bases, GTG, of one strand; then the spermine winds across the groove to interact with the corresponding set of bases on the other strand. The methylene groups of spermine form a hydrophobic cluster with the methyl groups of the thymines and the O6 atoms of the guanines of the TGT sequences on either side of the dyad. The observed mode of binding of spermine to A-DNA can serve as a model for deep groove binding in RNA and DNA–RNA hybrids that show a propensity also for the A-conformation. It will be of interest to see if base binding of spermine to DNA is involved in the regulation of gene expression, since spermine and other oligocations are ubiquitous in cells and their concentration is coupled to stages in cell cycle.

Herein we report the crystal structure of the complex of the oligocation spermine [[*N*,*N*′-bis(ammoniopropyl)diammonio]butane, $NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$] and the DNA octamer d(GTGTACAC). The octamer sequence was initially chosen to understand the structural features that may be unique to the alternating $d(TG)_n/d(CA)_n$ tracts that are ubiquitous in eukaryotic systems. Long stretches of such sequences have been found in introns (Hamada et al., 1982) while shorter stretches commonly occur in genetic regulatory regions such as the flanking sequence of the SV 40 enhancer (Nordheim & Rich, 1983). Our crystallographic study reveals that the octamer adopts a right-handed A-DNA structure, and the GT/CA block engages in a novel cross-strand bifurcated hydrogen bonding between the purines G and A. Although the A-form is not known to be present in the genomic DNA, it is believed to be the exclusive conformation for RNA and DNA–RNA hybrids. In the complex, the spermine is bound to the floor of the deep major groove of the A-DNA where it interacts only with the bases and not with the phosphates. In a previous crystallographic study at 1.9-Å resolution, spermine was found to bind across the major groove of a B-DNA dodecamer, interacting with the phosphates of the opposite strands and with a guanine base (Drew & Dickerson, 1981). In a lower resolution study of the structure of yeast phenylalanine tRNA, spermine was found to bind similarly to phosphates and bases across the deep groove (Quigley et al., 1978). Solution studies have implicated a diffuse nonspecific mode of binding of spermine to the anionic phosphate backbone of the DNA (Braunlin et al., 1982; Wemmer et al., 1985). Thus our observation is unexpected and is of interest in view of the fact that spermine occurs naturally in eukaryotic cells and its concentration increases dramatically just prior to the synthesis phase of the cell cycle (Tabor & Tabor 1984).

Materials and Methods

The self-complementary octamer d(GTGTACAC) was crystallized in the presence of spermine and $MgCl_2$. The crystals belong to the tetragonal space group $P4_32_12$, and the cell constants are $a = b = 42.43$ Å and $c = 24.75$ Å. The intensity data were collected by the oscillation film method using the Elliot GX6 rotating anode X-ray source (Jain et al., 1987). Initially, the 2.5-Å data set was collected with a short exposure time of 1500 s/deg, and then the 2.0-Å resolution data set was collected with a longer exposure time of 5000 s/deg, providing 1365 unique observed reflections. The preliminary structural solution was obtained at 2.5-Å resolution (Jain et al., 1987), and the structure was subjected to 20 rounds of refinement. Each round consisted of fitting the structure to $2F_o - F_c$ and $F_o - F_c$ omit maps on the Evans and Sutherland graphics system using the program FRODO (Jones, 1985) and then performing several cycles of restrained least-squares refinement with the program of Hendrickson and Konnert (1979), as modified for nucleic acids (Westhof et al., 1985). Subsequently, the data were slowly extended out to 2.0-Å resolution, and nine more rounds of refinement were performed with 1214 reflections between 5.0 and 2.0 Å. The data below 5.0-Å resolution were excluded because they were heavily influenced by bulk solvent scattering.

The difference maps obtained very early in our 2.5-Å work showed a stretch of electron density that was suspected to be

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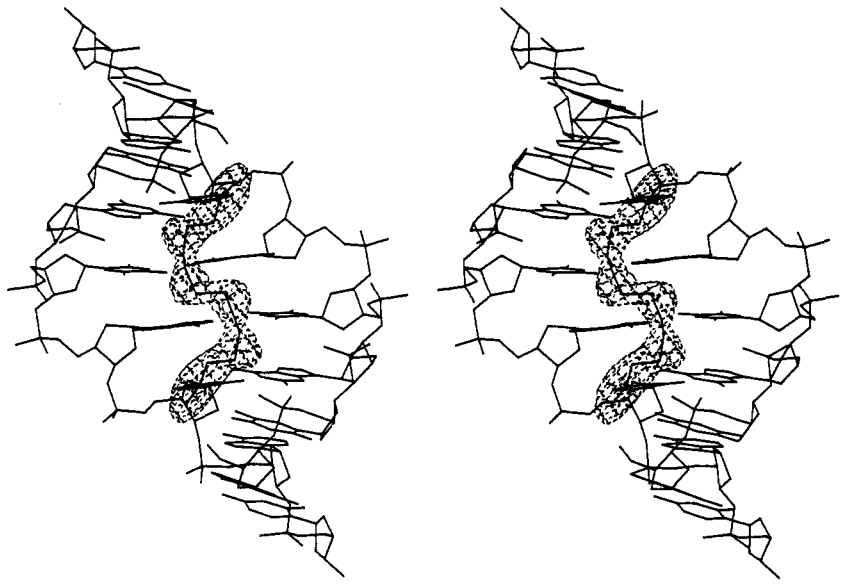

FIGURE 1: Stereopair showing the difference electron density for the spermine, contoured at 2.7 σ. The density was calculated after omitting the spermine from the final model.

Table I: Helical and Base-Pair Parameters for d(GTGTACAC)[a]

base pair	Φ_{tilt} (deg)	Θ_{roll} (deg)	prop. twist (deg)	buckle (deg)	bp displ (Å)	helical twist (deg)	rise/residue (Å)
G-C	11.5		13.5	0.5	3.15		
		6.0				31.3	3.51
T-A	9.1		9.9	−2.8	3.46		
		10.8				31.3	3.19
G-C	9.2		13.7	−1.8	3.27		
		6.4				36.0	3.05
T-A	10.4		9.2	−3.3	3.47		
		5.6				28.3	3.13
A-T							

[a] See Fratini et al. (1982) for definitions.

a spermine site in the deep groove of the DNA. Initially this density was fitted by a string of six water molecules. As the refinement progressed, a complete density for spermine became visible, which was then fitted with a spermine (Figure 1). Other waters were also incorporated on the basis of the presence of a globular density at 2 σ level within 2.2–3.6 Å from a hydrogen-bond donor or acceptor atom. The isotropic thermal parameters and occupancies of waters were refined in succession. In the final cycles, 43 waters, half of the spermine molecule, and one strand of the octamer formed the asymmetric unit and were refined. The *R*-factor for the 1214 reflections between 5.0- and 2.0-Å resolution was 0.115. The bond lengths and angles of non-phosphate groups have rms deviations of 0.01 Å and 1.7°, respectively, from standard values, indicating that the structure has sound stereochemistry. The coordinates have been deposited in the Brookhaven data bank.

Results and Discussion

The Kinked A-DNA. The alternating Pu-Py (Pu, purine; Py, pyrimidine) sequence GTGTACAC, with a 5′-Pu start, adopts a right-handed A-DNA conformation, whereas all of the known alternating Py-Pu sequences, with a 5′-Py start, adopt the left-handed Z-DNA conformation. As discussed in our earlier paper, the 5′-Pu start leaves more Pu-Py stacks than Py-Pu stacks, thus shifting the stacking interactions in favor of a right-handed structure (Jain et al., 1987; Quadrifoglio et al., 1984). The extra-Watson–Crick hydrogen bonds and spermine binding may further stabilize the right-handed conformation. Although the backbone torsion angles of the molceule are generally in the standard ranges for an A-DNA, the central T4A5/A13T12 base-pair step exhibits conformational distortions in the backbone from the usual A-structure. The A5 and A13 bases display trans rather than the usual gauche conformations around the C4′–C5′ (186°) and the P–O5′ (141°) bonds (Figure 2, top). This feature has also been observed in the Py-Pu step of other octamer structures that crystallize in the tetragonal space group (Heinemann et al., 1987). We note that a consequence of this distortion is a kink at the site and a resultant opening up of the major groove. The GTGTACAC duplex is bent at the dyad axis toward the shallow groove by an angle of 12°, and its deep groove width has increased to 8.7 Å.

The helical and base-pair parameters (Fratini et al., 1982) for the octamer, such as the roll, propeller twist, helical twist, and rise/residue, are shown in Table I. The propeller twist and the helical twist show an underlying alternation, following the alternation in the sequence. However, this trend is not seen at the ends, due to end effects. It is also noteworthy that the propeller twist angles for the G-C base pairs (average 13.6°) are significantly higher than those for the A-T base pairs (average 9.6°), opposite of what is seen in the other known oligonucleotides.

Extra-Watson–Crick Hydrogen Bonds. An interesting feature of the structure is the presence of extra-Watson–Crick cross-strand hydrogen bonds between the purines G3-A13 and A5-G11, in addition to the usual Watson–Crick (WC) base pairing, in the central region of the octamer (Figure 3). In other words, the N6 amino proton of adenine A13 (A5), besides forming the normal WC hydrogen bond to the O4 car-

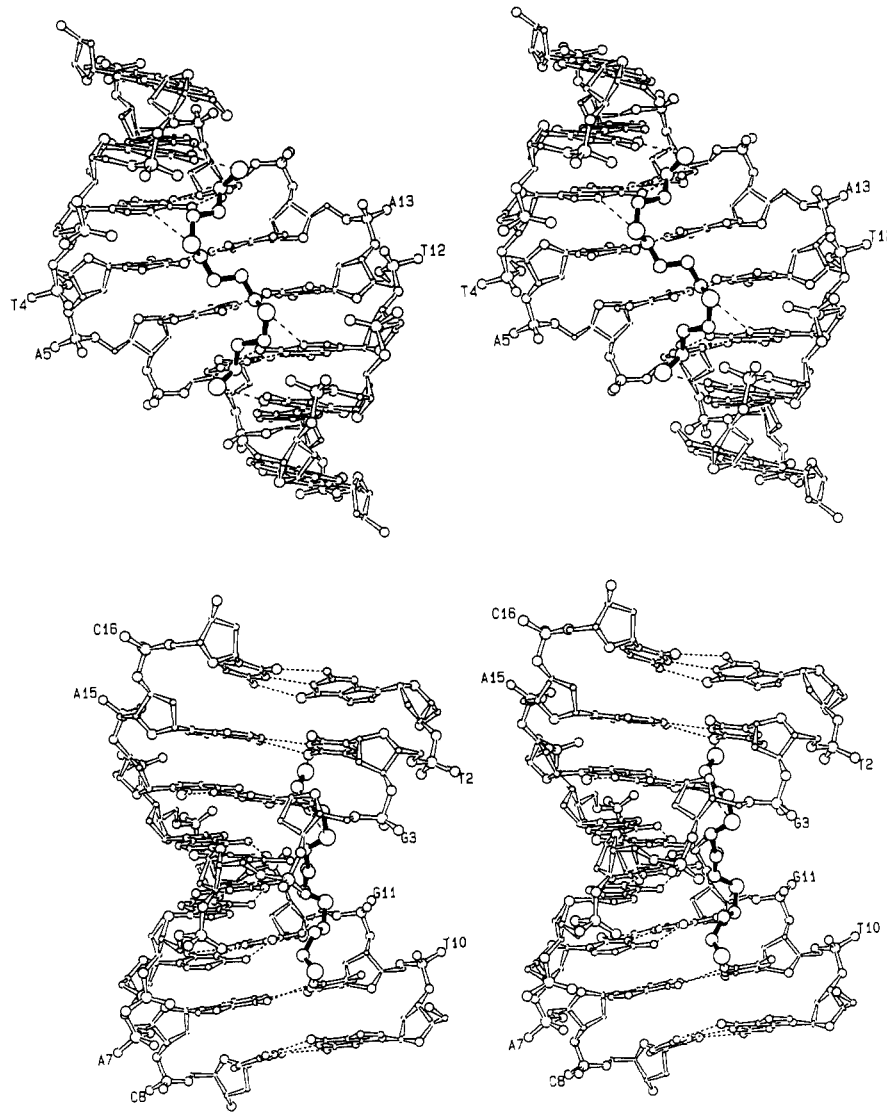

FIGURE 2: Stereopairs of the spermine d(GTGTACAC) complex: (top) viewed into the deep groove down the dyad axis; (bottom) viewed perpendicular to the dyad that lies in the plane of the paper.

bonyl of thymine T4 (T12), is also involved in a hydrogen bond with the O6 atom of the guanine G3 (G11), in the adjacent base pair. The distances and angles involving the protons are given in the caption to Figure 3. Thus, the adenine proton is involved in a three-center hydrogen bond (Jeffrey & Mitra, 1984). The propeller twisting of the base pairs, which apparently occurs to maximize intrastrand base stacking (Calladine, 1982), also assists in the formation of the three-center hydrogen bonding at the center of the octamer. In other words, while base-pair skewing is natural for DNA to maximize intrastrand base stacking, three-center hydrogen bonding may occur if the appropriate donor–acceptor base oppositions are present in the adjacent base-pair steps, and this would amplify the propeller twisting.

Calladine's rules (Calladine, 1982) state that, in an alternating DNA structure, propeller twisting causes the purines on the opposite strands to exhibit steric clash in the major groove for a 5′-Pu-Py/Py-Pu sequence and in the minor groove for a 5′-Py-Pu/Pu-Py sequence. His rules provide mechanisms through which these clashes could be relieved in the B-DNA. These rules have been extended to A-DNA as well with some success (Dickerson, 1983) and are valid for A-A and G-G, but not for A-G, oppositions as in the octamer, where extra-WC hydrogen bonds are formed between the G3-A13 and A5-G11. To juxtapose the interacting atoms for three-center hydrogen bonding, the central GT/CA steps exhibit a high helical twist (36°) angle (Table I). On the other hand, due to end effects, there are no three-center hydrogen bonds between G1-A15/G9-A7 at either ends, which exhibit a low helical twist (31.3°) angle, consistent with Calladine's rules.

Recently Pu-Py (A-T) three-center hydrogen bonds have been observed in the major groove of the structures containing oligo[d(A)/d(T)] tracts, which are known to cause bending in B-DNA (Nelson et al., 1987; Coll et al., 1987). Our octamer structure has revealed that the extra Pu-Pu hydrogen bonds can also occur in another class of biologically important DNA tracts, oligo[d(GT)/d(CA)] (Figure 4). It appears that the Pu-Pu extra hydrogen bonds are also compatible with B-DNA, especially since B-DNA has a higher helical twist angle than A-DNA (36° versus 31.5°). The continuous network of three-center hydrogen bonding has been proposed to lead to rigidity in the oligo[d(A)] tract (Nelson et al., 1987). In the alternating oligo[d(GT)] tracts, however, three-center hydrogen bonds can occur only in the Pu-Py steps, but not in the Py-Pu steps. Consequently, the oligo[d(GT)] tracts may not be as rigid as the oligo[d(A)] tracts, but may still be more rigid than sequences without the three-center hydrogen bonds. Thus, these sequences may modulate the distensibility of DNA and provide punctuation marks for folding and packaging of DNA in nucleosomes.

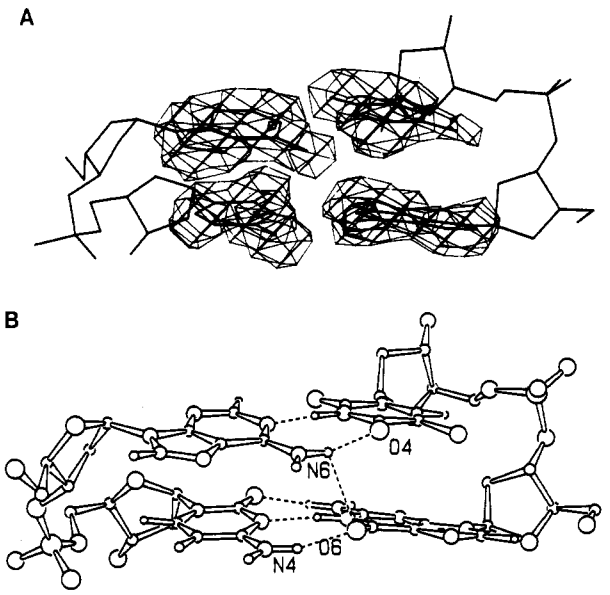

FIGURE 3: (A) "Omit" difference electron density map, contoured at 3.0 σ, for the four bases, G3-C14 and T4-A13, viewed from the deep groove side. (B) The extra-Watson–Crick hydrogen bond between N6 of A13 and O6 of G3 is shown. The distances from the N6 proton of adenine A13 (A5) to the O4 of thymine T4 (T12) and to the O6 of guanine G3 (G11) are 1.9 and 2.6 Å, respectively. The N–H···O hydrogen-bond angles are 157° for thymines and 116° for guanines, and the O···H···W angle is 87°. The values meet the criteria of Jeffrey (1984) for a three-center hydrogen bond.

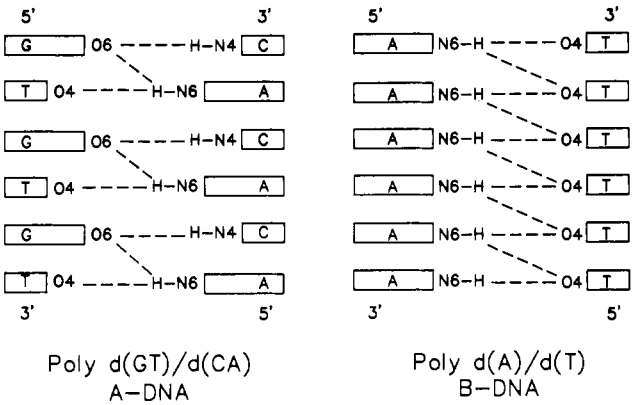

FIGURE 4: The deep groove (A-DNA) three-center hydrogen bonding in the d(GT) tracts and the broad groove (B-DNA) three-center hydrogen bonding in oligo(A) tracts.

Spermine Recognition of DNA. The most unexpected feature of the octamer structure is the sequence-dependent binding of spermine to the floor of the deep groove of the A-DNA. The spermine is located on the molecular dyad of the DNA and assumes roughly a helical "S" shape by adopting gauche conformations about the bonds C4–N5 (C9–N10) and C6–C7 (C8–C9) (Figure 2). The ammonium groups, N1 and N5 on the top half of spermine, form hydrogen bonds with the carbonyl O4 of T2 and imino N7 of G3, respectively (Figure 5). The serpentine molecule then spirals down the DNA helix and latches on to T10 and G11 of the symmetry-related opposite strand in an identical manner. Besides the hydrogen bonding, the complex exhibits numerous hydrophobic interactions between the methylene groups of the spermine and the base atoms on the floor of the major groove. In particular, the four central butane methylenes of spermine make van der Waals contacts with the methyl groups and O4 atoms of the thymines T4 and T12. The propane methylenes on either side of spermine make van der Waals contacts to O4 and C4 atoms of thymine 2T and T10 and to O6 and N7

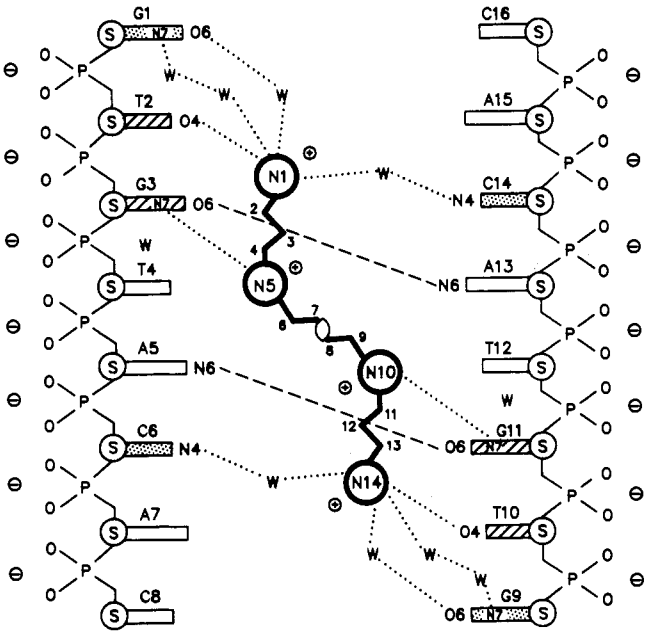

FIGURE 5: Schematic drawing showing the hydrogen-bonding interactions between the spermine and the octamer duplex. The striped and dotted bases, respectively, represent the direct and indirect (through waters) interactions with spermine. The bases T2 and G3 at the 5'-end are involved in direct hydrogen bonding to the spermine, while the bases G1 and C14 are involved through water bridges to the spermine. The central GTAC block is held "rigidly" by the cross-strand three-center hydrogen bonds.

atoms of guanine G3 and G11. These hydrophobic interactions must also contribute significantly to the binding energy of the spermine–DNA complex. There are also hydrogen-bonding interactions via water bridges between the terminal ammonium groups of spermine and the terminal guanines G1 and G9 (O6, N7) and cytosines C14 and C6 (N4) of the opposite strand.

Spermine binds to the same deep groove where the three-center hydrogen bonds occur. In contrast, the narrow groove binding drugs netropsin and distamycin bind specifically to the oligo[d(A)] tracts on the opposite side of the major groove Pu-Py three-center hydrogen bonds of the B-DNA (Kopka et al., 1985; Coll et al., 1987).

A surprising feature of the structure is the lack of any direct interaction of spermine with the phosphate groups of the DNA. Nevertheless, the deep groove binding of spermine found in our structure is consistent with the high electronegative potential in the deep groove of the A-DNA (Lavery & Pullman, 1981) generated mainly by the flanking backbone phosphates and the electronegative patch of atoms on the GTGT bases (O6, N7 of the G's and O4 of T's). Our structure provides another possible mechanism by which spermine can elevate the T_m of the DNA duplex by cross-strand interactions to the bases.

The spermine molecule spans the entire length of the eight base pairs of the octamer through hydrogen bonding, but the segment involving direct interactions with the bases is confined to the stretch of the central six base pairs, TGTACA. Such recognition lengths are common in resriction sites. Spermidine can bind in the same way as spermine, but can extend only to N7 of G11, thus breaking the 2-fold symmetry. However, in crystals, the crystallographic dyad may still be maintained with orientational disorder, where the aminopropyl moieties of spermidine would have half-occupancies.

The counterpart to the unusual base only binding mode of spermine to DNA, in contrast to the expected nonspecific binding to the phosphate backbone, was recently found in the

trp repressor binding to the operator DNA, involving only backbone phosphates instead of the expected base-specific binding (Otwinowski et al., 1988).

Spermine Binding to B-DNA, DNA–RNA Hybrids, and RNA. The present binding of spermine to DNA of the second kind (A-DNA) suggests a similar pattern of binding to RNA duplexes and DNA–RNA hybrids that form helices only of the second kind. The ribose O2′ atom in RNA is directed toward the minor groove and should not affect binding in the major groove. However, the absence of the methyl groups on uracil may weaken the hydrophobic interaction of the complex.

The present base only mode of binding of spermine does not seem possible for the same sequence in the B-DNA conformation. This is because the distance between the central nitrogens N5 and N10 of spermine, even in its fully extended conformation, is too short (5.8 Å) to bridge the N7's of the two purines spaces 11.6 Å apart by the two base-pair steps in the middle. However, because of the flexibility of the interacting molecules, other base-specific modes of binding may be possible, and differences in the binding energy of spermine in different binding modes could also induce conformational transitions in DNA.

Biological Implications. Polyamines are required for normal growth of all cells tested, and spermine is the most abundant polyamine found in animal cells (Tabor & Tabor, 1984). Nevertheless, the precise biological role of polyamines remains elusive. It has been proposed that they may serve the function of condensing nucleic acids through neutralization of negative charges on the phosphates (Gosule & Schellman, 1976). However, some observations suggest that polyamines may also have some regulatory role in the synthesis of biological macromolecules. For example, in mammalian cells, a surge in polyamines and specifically spermine precedes an increase in the content of DNA, RNA, and protein during a growth spurt (Tabor & Tabor, 1984). Ornithine decarboxylase that catalyzes the rate-limiting steps in the biosynthesis of polyamines has a very short half-life and is feedback regulated by the concentration of polyamines at the translational level (Person et al., 1986). Polyamines are also known to enhance the activity of several DNA-binding proteins such as DNA polymerase α (Fisher & Korn, 1979). These potentially regulatory functions of polyamines may involve specific binding of polyamines to certain sequences, as well as to the phosphates, in the nucleic acids. At present there is only limited information on the potential role of spermine in modulating the expression of genes related to cell growth or proliferation, and further study is required.

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