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A new peculiar DNA structure: NMR solution structure of a DNA kissing complex

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Abstract

The deoxyoligoribonucleotide d(CTTGCTGAAGCGCGCACGGCAAG) (dSL1) corresponding to the reverse transcribed sequence of the dimerization initiation site SL1 of HIV-1Lai RNA was synthesized using phosphoramidite chemistry. Like its oligoribonucleotide counterpart, dSL1 dimerized spontaneously in solution. Here we report the first NMR solution structure of a kissing complex formed with two DNA strands. The melting point of the DNA dimer (35 °C) was found slightly higher than the one of the corresponding RNA dimer (32 °C). Despite this only slight difference in melting point, several structural differences were observed between the ribo- and the deoxyribo- dimers. The solution structure of the deoxy- dimer was a symmetric homodimer with a loop-loop interaction stabilized by four central G-C base-pairs, a head to tail A-A base-pair arrangement between the A8 residues of the two strands and a stacking of A9 with C15. As a consequence, G10 was not paired and occupied a position outside the stem and the loop. Each stem was formed by seven basepairs whose axis made an angle of about 100° with the plane of the loops. The distortion of the helix at the junction of the stem and of the loop induced a fold up of the A8pA9 step with a phosphate-phosphate distance lowered to 4.5Å. The plane of the non-canonical A-A basepair was oriented perpendicularly to the axis of the stems. The four central base-pairs formed an open fan-shaped motif with an angle of 20° between the bases and each of them was oriented perpendicularly to the A8-A8 plane. The deviation of the computed chemical shifts and the experimental ones for the aromatic proton was always less than 0.25ppm for each of the 16 converged solution structures and their average less than 0.1ppm.

Introduction

Hairpin structures formed in nucleic acids have received a considerable interest since they are implicated in important biological processes. However, if the RNA hairpins begin to be extensively known due to their implication in intra-RNA recognition processes (1-16) or in site specific interactions with numerous proteins with RNA (17), only little is known about the DNA hairpin structures.

Such structures are known as potential interaction sites for numerous proteins : topoisomerase II (18,19), HIV-1 integrase (20) and SMC (21). The DNA hairpins could play also a role in some neurodegenerative diseases (22) by interfering with the DNA polymerase slippage (23).

Since in our laboratory, we had focused attention on hairpin structures and conformational changes associated with the dimerization of retroviral RNA, we became interested in checking if such types of structures were possible with deoxyribonucleotides since the proviral DNA contains on one strand sequences which are equivalent to those of the genomic RNA.

Our previous biochemical and NMR work together with many studies from other laboratories have shown that a sequence localized immediately upstream of the splice donor site inside the HIV-1 Psi-RNA region is responsible for the dimerization initiation of the retroviral RNA. This 35 bases long sequence which is called SL1, is highly conserved and can adopt a stem loop structure which presents a complementary sequence in the loop. It has been shown that in vitro HIV-1 RNA dimer formation could be initiated by the annealing of the loop self-complementary sequence GCGCGC through a loop-loop recognition (13).

Figure 1: Tentative scheme of two possible conformations of the self-associated dSL1: the kissing complex (top) and the extended duplex (below) on the basis of the two structures obtained with the dimer of SL1 HIV-1 wild type.

Along the dimerization process, this loop-loop duplex is converted either through temperature or through nucleocapside protein (14-16,24) into a more stable extended duplex. Structure of a kissing complex formed from a slightly mutated RNA fragment has already been proposed (25). Furthermore the solution structure of the extended duplex was elucidated (26) and a model corresponding to the transition from the loop-loop complex toward the extended duplex has been described in details (27) leading to the conclusion that the central stretches of G-C base pairs in the loop were kept associated during the transition.

Nevertheless, although the three dimensional solution structure was experimentally studied, the role played by the hydroxyl groups 2'OH has never been investigated. Very recently, the analysis of a 10 ns dynamics trajectory including water molecules and carried out on a mutated SL1 RNA sequence where G12 has been substituted by A12 to prevent dimerization, indicated the formation of a stabilizing belt

of hydrogen bonds between adjacent nucleotides both in the stem and the loop involving the 2'OH groups (to be published).

In order to understand the implication of these 2'OH on the formation and the stabilization of a stem-loop structure together with the formation of a loop – loop duplex, we present here structural results obtained with 23 nucleotides long DNA sequence: C248 – G270 (dSL1) .

The primary question was to know if such a sequence was able to form a stem-loop structure and furthermore to investigate if a DNA hairpin comprising a selfcomplementary sequence in the loop would be able to form a loop – loop duplex.

We present here the NMR solution structure of a loop – loop duplex obtained from SL1DNA sequence. This loop – loop structure presents several different features than the one obtained from equivalent SL1 RNA and these differences are discussed.

Materials and Methods

Sample preparation and folding

The 23-mer DNA (Figure 1) was synthesized on an automatic Millipore Expedite with the phosphoramidite chemistry. Two CPG columns of 15 micromoles were used. The DMT-on oligonucleotide was purified on reverse phase HPLC. After detritylation, the crude product was purified on HPLC, first with an ion-exchange (Waters DEAE-8HR) then with a reverse phase (Nucleosil 300-5C18) column. The total yield, after ion exchanging with Dowex-Na⁺ , was 10.4% (23.5mg).

The sample was passed through a Chelex 100 column in order to remove the paramagnetic impurities for NMR experiments. The DNAsample was refolded by heating to 90 °C during 3 minutes then snap-frozen at 4 °C for 4 minutes. The sample was concentrated by lyophilization and then dissolved in 90% H₂O / 10% D₂O or 100% D₂O argon-degassed solution at a concentration of 2mM in a phosphate buffer 10mM at pH 5.8. An alternative protocol where the sample was folded by slow decreasing of the temperature to 90 to 4 °C led to NMR spectra of different features which will be published elsewhere.

NMR experiments

NMR experiments were carried out on a Unity INOVA 600MHz spectrometer operating at 14.09 Tesla. Spectra were recorded with 300 and 1024 complex data points in the t1 and t2 dimensions. All D₂O experiments water were carried out with the carrier frequency set on the H₂O resonance, and a sweep width of 5997 Hz in the F1 and F2 dimensions. The residual H₂O resonance was irradiated by a weak presaturation during 1s. For experiments in H₂O, a sweep width of 13000 Hz was used in the F1 and F2 dimensions. The watergate sequence (28) was used in order to remove the signal of water. Two-dimensional data sets for DQF-COSY, TOCSY and NOESY were recorded in the States mode. In D₂O five NOESY spectra corresponding to 0, 50, 90, 150 and 300ms mixing time were collected at 27 °C with 32 scans for each T1 value and a recovery delay of 2s without removing the sample or changing the gain. The t1 data were zero-filled to 2048 points and processed with a sine 90 phase shifted function. This apodization function does not distort the signal intensities, and the resulting peaks have no truncation effects. Correct phasing of the data in both dimensions was achieved with great care in order to quench the zero-quantum contribution in the cross-peak integration. The NOE values were obtained by dividing the volume of the cross-peak by the corresponding value of the diagonal obtained at 0ms mixing time. The cross-peak volumes were calculated with integration routines of the VNMR software package. The 2D 1H-31P correlation spectra

were recorded at 27 °C with 480 scans and with 48 and 1024 complex data points in the t1 and t2 dimensions, a sweep width of 5997 Hz and 800Hz for 1H and 31P and a repetition time of 1s. All 1H and 31P spectra were referenced to the resonance from DSS and trimethylphosphate respectively.

Results

Spectral assignments

The NOE patterns for the stem region of dSL1 were consistent with those of a righthanded B-type helix. Figure 2 shows the NOESY spectra of the 23-mer DNA recorded in D₂O. The seven H5-H6 connectivities as well as the three CH3-H6 connectivities observed on the TOCSY spectrum were good starting points for the sequential assignment. At 300ms mixing time, all the H6/8-H1', H6/8-H2'/2'' and H6/8-H6/8 intra or inter connectivities were observed, leading to a total unambiguous sequential assignment at 27 °C. Figure 3 shows the NOESY spectra obtained at 120ms mixing time in H₂O : D₂O (90:10) at 15 °C. The sequential assignment of the imino and amino protons in the stem was made directly and verified by comparison with the H5-H6 or CH3-H6 connectivities in D₂O. The assignment of the exchangeable protons of the 3 central G-C base-pairs was obtained starting to the H5 and H6 assigned resonances in D₂O. No connectivity implying the exchangeable protons of G10 or C15 was observed for temperature between 4 °C and 40 °C. A connectivity between H8(A8) and a NH2 resonance at 7.80ppm was observed. It was tentatively assigned to an amino proton of the adenine A8 or A9 or A16 since the amino groups of A21 and A22 were already assigned.

Figure 2: Expansion of the aromatic to H1' proton region of the NOESY spectrum of dSL1 dimer in D₂O at 27 °C obtained with a mixing time of 300ms. We can observe the very weak connectivity H1'(G7)-H8(A8), the medium connectivities H2(A8)-H1'(G14), H2(A8)-H5(C15) and H2(A9)-H6(C15) and the lack of connectivity between H8(G14) and H5(C15). The arrows show the H2 resonances of the adenines A8, A9 and A16.

Usual and unusual NOEs

Several special features were characteristic of the structure of this sequence in this conformation. The guanine of G10 as well as the cytosine of C15 were found unpaired. The observed NOE between H8(A8) and NH2(A8, A9 or A16) suggested the presence of an A8-A8 or a A9-A8 or a A16-A8 base-pair since the distance between H8 and

its own nearest amino proton (4.9 Å) is too large to induce a NOE of medium intensity. The high field chemical shift of the H8(A8) proton (8.42ppm) reflected the lack of stacking for the five membered ring of the corresponding adenine. NOE data showed that the H2 of A8 was near to H1'(G14), H5(C15) and that the H2(A9) was near to the H1'(C15) although A8 and A9 are far away from G14 and C15 in the

sequence. Moreover, H2(A8) was found near the H8(G14) and the H2(A16). The upfield chemical shift of H6(C15) as well as the NOEs H6(C15)-H2(A8) and H6(C15)-H2(A9) suggested the stacking of C15 between A8 and A9.

Figure 3: Portions of the 600MHz NOESY spectrum for the dSL1 dimer in H₂O: D₂O (90 :10) solution at 05 °C obtained at 300ms mixing time. a) connectivities between the imino protons b) connectivities between the imino protons and the aromatic or the amino protons.

Duplex and backbone conformation

The folding of dSL1 can be predicted from the NOESY and the COSY-DQF data. Watson-Crick base-pairing, weak intensities of H8/6(i)-H1'(i), strong intensities of H8/6(i)-H2'(i) and predominately C2'-endo sugar conformations all together indicate that the duplex, excepted A8, A9, G10, C15 and A16 presents a B-DNA helical structure. Moreover, the 1H-31P correlation spectrum of dSL1 shown

in Figure 4, the majority of the resonances are concentrated in a narrow chemical-shift range of 1.0 ppm, a standard for regular helices. However, four resonances are found out of this range. Two of them correspond to G7pA8 and A8pA9 step and reflect the backbone distortion at the hinge between the stem base-pairs and the central basepairs. The two other resonances were assigned to C13pG14 and G14pC15.

Figure 4: ³¹P HSQC spectra of the dSL1 dimer obtained at 27 °C. The connectivities of the ³¹P resonances with H3' and H4' for the phosphorus G7pA8, A8pA9, C15pA16, A16pC17 and C17pG18 are indicated.

Structure determination

Experimental constraints

Figure 5 shows a diagram summarizing the internucleotide NOE network observed for the residues G7 to C17 in the NOESY spectra of the DNASL1 dimer. Since evidence of chemical exchange between

the same proton of the two strands was never observed in the NMR spectra, the same constraints were introduced for the corresponding nucleotides of each strands but the annealing process was never constrained to give a symmetrical structure. A total of 684 distance restraints, 258 dihedral restraints and 102 hydrogen bonding distances were used for the structure calculations (Table 1). This corresponds to 15 experimental constraints by residue.

A C2'-endo conformation was assigned to all the ribose rings based on the J coupling constants observed on the DQF-COSY spectrum and was achieved by restraining their endocyclic torsion angles. For the other five backbone dihedral angles, a set of loose B-form helix restraints (B-form backbone $\pm 15^\circ$) were used to constrain the stem residues backbone (C1 to G7 and C17 to G23) and the C11-G14 sequence to adopt a right helix conformation.

Table 1: NMR restraints and statistics for the dSL1 kissing complex. Data into brackets indicate values obtained with the dSL1 extended duplex structures. (1) Geometric terms included bond, angle, improper, NOE and dihedral terms. (2) R.M.S.D : root mean square deviation in positions of heavy atoms compared to the global average structure.

NMR restraints			
Distance restraints from D ₂ O data			
Intra-residue		172	
Inter-residue		322	
Distance restraints from H ₂ O data			
Watson-Crick base pairing		102	
Exchangeable		88	
Dihedral restraints			
Sugar pucker (ν_1, ν_2, ν_3)		138	
Backbone		120	
Total distance restraints		684	
Force constant		50 kcal.mol ⁻¹ Å ⁻²	
Distance restraints per residue		14.9	
Total dihedral restraints		258	
Force constant		50 kcal.mol ⁻¹ rad ⁻²	
Dihedral restraints per residue		5.6	
Total restraints		942	
Distance and dihedral restraints per residue		20.5	
Structural statistics			
Number of structures included		17	(5)
Energy			
All terms CHARMM force field calculation	- 613 ± 70 kcal.mol ⁻¹	(+13819±1650 kcal.mol ⁻¹)	
Geometric terms ⁽¹⁾	+ 309 ± 47 kcal.mol ⁻¹	(+11923±1270 kcal.mol ⁻¹)	
Van der Waals term	- 531 ± 18 kcal.mol ⁻¹	(-2172±512 kcal.mol ⁻¹)	
Electrostatic term	- 391 ± 25 kcal.mol ⁻¹	(-276±7 kcal.mol ⁻¹)	
NMR constraints violation			
Number of distance violation up to 0.5 Å	0	(27±4)	
Number of distance violation up to 0.3 Å	3 ± 2	(105±7)	
Number of dihedral violation up to 5°	0	(4±2)	
R.M.S.D ⁽²⁾			
Stem 1 (bases:1-7;17-23)	0.44 ± 0.17 Å	(9.2±2.8 Å)	
Stem 2 (bases:1'-7';17'-23')	0.41 ± 0.20 Å	(8.1±1.9 Å)	
Middle stem (bases:11-14;11'-14')	0.32 ± 0.11 Å	(2.3±0.7 Å)	
Junctional bases (8,9,10,15,16;8',9',10',15',16')	1.11 ± 0.35 Å	(7.7±1.3 Å)	
Whole duplex	1.72 ± 0.58 Å	(10.5±2.8 Å)	

Structure

It has been shown that the RNA SL1 exists under two conformations in solution: the kissing complex and the extended form (25-27). It is not possible to discriminate between these forms on the basis of qualitative NOE data only. Thus, molecular dynamics calculations were undertaken with two methods in order to distinguish between the two types of associations (Figure 1). The first method fixed a linear duplex form i.e. pairing C1 with G23 of the other strand, pairing C11 with G14 of the other strand and so on. The second way fixed a kissing complex conformation, i.e. the pairing of C1 with G23 of the same strand, the pairing of C11 with G14 of the other strand and so on. The choice between the two conformations was made on the basis of the NOE energy and more importantly on the number of distance violations implicating the residues A8, A9, G10, C15 and A16.

In both cases, one hundred random structures were generated using CNS 1.0 (29). These basic starting structures were subjected to a simulated annealing-restrained molecular dynamic protocol in torsion angle space with a force-field consisting of bond lengths, bond angles, improper angles, repulsive van der Waals potential (single quartic repulsion), experimental distances and dihedral angles constraints from the NMR data (30). The force constants

were imposed to be 50 Kcal.mol⁻¹.Å⁻² for all distances and 50 Kcal.mol⁻¹.rad⁻² for dihedral terms. The electrostatic terms were not present during the simulated annealing. Very close non-bonded contacts were first removed by 200 steps of Powell minimization (31). Initial velocities were assigned according to a Maxwell distribution at 20000K. Then an equilibration period consisted in 15ps dynamics in torsion angle space at 20000K with a Van der Waals scale factor of 0.1. The first cooling stage consisted in 75 ps dynamics in torsion angle space where the temperature was cooled down from 20000K to 0K with the Van der Waals scale factor increased from 0.1 to 1. This protocol was followed by a second cooling stage which consists in 80 ps dynamics in cartesian space where the temperature was cooled down from 2000K to 0K and where the Van der Waals scale factor increased from 1 to 4. This protocol was finally followed by 1000 steps of Powell minimization with full electrostatic and Van der Waals terms. At this point, the converged structures were selected on the basis of their low NOE violations and their total energies. Finally, the structure convergence was examined graphically.

Figure 5: Summary of the distance restraints for the sequence G7-C17 / G7'-C17'. The observed interstrand restraints between the protons of the residues A8 and A8', A9 and C11 as well as the intrastrand connectivities between the non adjacent A8 and A9 with C15 or A16 residues are indicated.

Loop-loop interaction in the dimer

The global average structure of the 16 structures consisted in a symmetric homodimer (Figure 6) in which both SL1 whole stems (C1 to G7 and C17 to G23) form canonical B-type helix. The loop-loop interaction was stabilized by four Watson-Crick base-pairs involving bases of the strands C11-G14 and G12-C13. The stemloop junctions were stabilized by head to tail A-A base-pairs involving two N6HN7 hydrogen bonds, a hydrogen bond between N6H(A9) and N3(A8) of the same strands and a stacking of the adenine A9 with C15 of the other strand. All the attempts to introduce a H8(A8)-NH2(A9 or A16) restraint failed whereas the distance between H8(A8) of a strand and NH2(A8) of the other strand was found 3.4Å even if this restraint is not specifically introduced. The guanine of G10 was found unpaired and out of the loops as already suggested by the lack of upfield shift of its H8 resonance. All the attempts to paired

G10 and C15 lead to substantial violations of several restraints. The distortion of the helix conformation at the hinge of the stem and the loop induced a fold up of the A8pA9 backbone through a gauche⁺-gauche⁻ transition of γ (A8) and a phosphate-phosphate distance of 4.5Å. The plane of the two paired adenines A8 was perpendicular to the axis of the stems. The planes of the central G-C base-pairs were perpendicular to the axis of the stems and of the planes of the adenines A8. The whole of the four G-C central pairs made a motif in open fan-shaped with an angle of 20 degrees between each plane, each plane been perpendicular to the A8-A8 plane (Figure 7).

Figure 6: Global view of the kissing complexes of dSL1 (left) and of the RNA type of SL1 HIV-1Lai from Mujeeb et al (25), PDB accession number 1bau(right).

Figure 7: Two orthogonal views of the A8 to C15 residues in the metastable kissing complex. The axis of the two stems are perpendicularly to the plane of the figure. The planes of the four central G-C base-pairs and of the two adenines A8 are perpendicularly to the figure. This motif is centered in the middle of the oligonucleotide, symmetrically to the edges. The noncanonical A8-A8' base pair, the stacking of A9 and C15' of the same strands and the

unstacked G10 residues as well as their orientations are well visible. In order to clarify the figure, the planes of the bases are only shown and the base pairs G14-C11', C13-G12', G12-C13' and C11-G14' are noted G14, C13, G12 and C11 respectively.

Test of the structure by back calculation of the chemical shifts

The 16 converged structures were tested by comparing the computed chemical shifts of the ¹H resonances by running NUCHEMICS (32) on each structure and the experimental ones. The maximum deviations of the computed chemical shifts from the experimental ones were 0.25, 0.20, 0.20ppm for the class of resonances (H6, H8), (H2, H5, CH3) and H1' respectively. The corresponding average of the absolute value deviations were always less to 0.1, 0.06 and 0.08ppm respectively. Moreover the chemical shift deviations of the resonances belonging to residues whose special surrounding induced strong upfield shifts such as H8(G7)(1.01ppm), H8(A9)(0.63ppm), H6(C13)(0.89ppm), H2 of all the adenines (0.82 to 1.23ppm), H1'(A8)(1.12ppm) were not higher than the others. We conclude that the special features of the unstable dimer of dSL1 were well represented by the 16 converged structures where only four G-C central base-pairs were observed. This last feature is a good test of the validity of our structure.

Comparison with the SL1 RNA kissing complex structure

The global average structures of both RNA and DNA of the SL1 kissing complexes in solution were a symmetric homodimer with loop-loop interaction. In both molecules, the structures of the kissing complexes are bent (about 100° between the axis of the stem and the plane of the loop) and metastable. Their interfaces were formed not only by standard base pairing between palindromes, but also by a distinctive pattern of interstrand interactions at the stem-loop junctions. The backbone of the residues A9-C17 has the shape of a nearly planar U with slightly convergent ends. Each loop is deflected toward the major groove of its own stem, and the stems converge toward the major groove of the helix. In both cases, the structure is marked by abrupt discontinuities of stacking and by several unpaired junctional bases because the defect of self-complementary in this region. Nevertheless, several differences between the structural features of the two kissing complexes can be pointed out. Kissing complex formation of RNA SL1 induced a distortion of the loop helix junction and ruptured the distal G7-C17 base-pair of end stem while in the DNA sequence the G7 is Watson-Crick paired with C17. On the other hand, in RNA the six central G-C base pairs are present in the loop-loop interaction whereas only four of the central base pairs are formed with the DNA sequence. Moreover in the DNA, the A8 residues of the two strands are hydrogen bonded while in the RNA the three adenines formed a pair of interloop triple-base arrays (A8-A9-C17) which are assumed to be critical for stabilizing the kissing complex. Consequently, in dSL1 the adenine of A9 was stacked with C15 in the same strand and the guanine of G10 was excluded of the stem and of the loop-loop motif. In RNA, A9 crossed the interface of its strand to stack with the A8 and C17 of the other strand. In the solution structure, this motif is found stabilized by an hydrogen bond between O5'(A9) and 2'OH(A8). On the other hand, the stems of the RNA and DNA were found near to canonical A-helix and B-helix as generally observed for RNA and DNA, respectively. Despite these differences, the stability of the RNA and DNA kissing complex were about the same (melting temperatures of 32 °C and 35 °C, respectively).

It can be pointed out that adenines are always present at the stem-loop junction and in the loop of different SL1 isolates (33). For example, three adenines (A8, A9 and A16) are present in the SL1 of Lai isolate, and two adenines and one guanine in the SL1 Mal isolate. In the kissing complex of SL1Lai, A8 and A9 are implied in a

triple base stacking with C17 of the other strand (25). In SL1Mal, the two adenines A8 and A16 were found in close proximity (34) suggesting a triple base-pair between bases of the two strands (33). In the extended duplex of SL1Lai, the three adenines A8, A9 and A16 form a zipper like motif involving the two strands of the duplex. In the metastable kissing complex of dSL1Lai, the adenines A8 of the two strands are paired and A9 is strongly stacked with C15. Although these adenines are implied in each case in different motifs, they are always participating as stabilizing factors of the two strand interaction. We conclude that, independently of the ribose or deoxyribose nature of the nucleic acid, the conserved adenines in the stem-loop junction play a key role in stabilizing the association of the strands.

Other factors such as hydrogen bonds involving the 2'OH hydroxyl groups in RNA, and bridges with water molecules can be important for stabilization but have not been investigated since the exchangeable OH resonances were not detected by NMR. Actually, dynamics molecular trajectories of the RNA kissing complex are run in order to study the stabilizing role of the 2'OH groups. Results will be published later on.

Coordinates

The PDB accession number for this structure is 1JUO

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Supplementary Material

This manuscript contains supplementary material. Table S1, is available at www.jbsdonline.com

References and Footnotes

1. Eguchi Y., Itoh T., and Tomizawa J. *Annu Rev Biochem* 60, 631-652 (1991).
2. Tomizawa J. *Cell* 38, 861-870 (1984).
3. Tomizawa J., and Som T. *Cell* 38, 871-878 (1984).
4. Tomizawa J. *Cell* 40, 527-535 (1985).
5. Tomizawa J. *Cell* 47, 89-97 (1986).
6. Tomizawa J. *J Mol Biol* 212, 683-694 (1990).
7. Coffin J. M. (1984) (Weiss, R., Teich, N., Varmus, H., and Coffin, J., Eds.) pp 261-368, Cold Spring Harbor, New York.
8. Bender W., and Davidson N. *Cell* 7, 595-607 (1976).
9. Temin H. M. *Proc Natl Acad Sci U S A* 90, 6900-6903 (1993).
10. Bieth E., Gabus C., and Darlix J. L. *Nucleic Acids Res* 18, 119-127 (1990).
11. Paillart J. C., Marquet R., Skripkin E., Ehresmann B., and Ehresmann C. *J Biol Chem* 269, 27486-27493 (1994).
12. Darlix J. L., Lapadat-Tapolsky M., de Rocquigny H., and Roques B. P. *J Mol Biol* 254, 523-537 (1995).
13. Muriaux D., Girard P. M., Bonnet-Mathoniere B., and Paoletti J. *J Biol Chem* 270, 8209-8216 (1995).
14. Laughrea M., and Jette L. *Biochemistry* 35, 9366-9374 (1996).
15. Laughrea M., and Jette L. *Biochemistry* 35, 1589-1598 (1996).
16. Muriaux D., Fosse P., and Paoletti J. *Biochemistry* 35, 5075-5082 (1996).
17. Gold L., Brown D., He Y., Shtatland T., Singer B. S., and Wu Y. *Proc Natl Acad Sci U S A* 94, 59-64 (1997).
18. Amir-Aslani A., Mauffret O., Bittoun P., Sourgen F., Monnot M., Lescot E., and Femandjian S. *Nucleic Acids Res* 23, 3850-3857 (1995).
19. El Amri C., Mauffret O., Monnot M., Tevanian G., Lescot E., Porumb H., and Femandjian S. *J Mol Biol* 294, 427-442 (1999).
20. Katz R. A., Gravuer K., and Skalka A. M. *J Biol Chem* 273, 24190-24195 (1998).
21. Connelly J. C., Kirkham L. A., and Leach D. R. *Proc Natl Acad Sci U S A* 95, 7969-7974 (1998).
22. McMurray C. T. *Proc Natl Acad Sci U S A* 96, 1823-1825 (1999).
23. Gacy A. M., Goellner G., Juranic N., Macura S., and McMurray C. T. *Cell* 81, 533-540 (1995).
24. Muriaux D., De Rocquigny H., Roques B. P., and Paoletti J. *J Biol Chem* 271, 33686-33692 (1996).
25. Mujeeb A., Clever J. L., Billeci T. M., James T. L., and Parslow T. G. *Nat Struct Biol* 5, 432-436 (1998).

26. Girard F., Barbault F., Gouyette C., Huynh-Dinh T., Paoletti J., and Lancelot G. *J Biomol Struct Dyn* 16, 1145-1157 (1999).
27. Theilleux-Delalande V., Girard F., Huynh-Dinh T., Lancelot G., and Paoletti J. *Eur J Biochem* 267, 2711-2719 (2000).
28. Piotto M., Saudek V., and Sklenar V. *J Biomol NMR* 2, 661-665 (1992).
29. Brunger A. T., Adams P. D., Clore G. M., DeLano W. L., Gros P., Grosse-Kunstleve R. W., Jiang J. S., Kuszewski J., Nilges M., Pannu N. S., Read R. J., Rice L. M., Simonson T., and Warren G. L. *Acta Crystallogr D Biol Crystallogr* 54, 905-921 (1998).
30. Stein E. G., Rice L. M., and Brunger A. T. *J Magn Reson* 124, 154-164 (1997).
31. Powell M. J. D. *Mathematical Programming* 12, 241-254 (1977).
32. Wijmenga S. S., Kruithof M., and Hilbers C. W. *J Biomol NMR* 10, 337 (1997).
33. Paillart J. C., Westhof E., Ehresmann C., Ehresmann B., and Marquet R. *J Mol Biol* 270, 36-49 (1997).
34. Dardel F., Marquet R., Ehresmann C., Ehresmann B., and Blanquet S. *Nucleic Acids Res* 26, 3567-3571 (1998).