High Affinity Interactions of Nucleolin with G-G-paired rDNA*

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Nucleolin is a very abundant eukaryotic protein that localizes to the nucleolus, where the rDNA undergoes transcription, replication, and recombination and where rRNA processing occurs. The top (non-template) strand of the rDNA is very guanine-rich and has considerable potential to form structures stabilized by G-G pairing. We have assayed binding of endogenous and recombinant nucleolin to synthetic oligonucleotides in which G-rich regions have formed intermolecular G-G pairs to produce either two-stranded G2 or four-stranded G4 DNA. We report that nucleolin binds G-G-paired DNA with very high affinity; the dissociation constant for interaction with G4 DNA is $K_D = 1 \text{ nM}$. Two separate domains of nucleolin can interact with G-G-paired DNA, the four RNA binding domains and the C-terminal Arg-Gly-Gly repeats. Both domains bind G4 DNA with high specificity and recognize G4 DNA structure independent of sequence context. The high affinity of the nucleolin/G4 DNA interaction identifies G-G-paired structures as natural binding targets of nucleolin in the nucleolus. The ability of two independent domains of nucleolin to bind G-G-paired structures suggests that nucleolin can function as an architectural factor in rDNA transcription, replication, or recombination.

Transcription and processing of rRNA occur within a specialized subnuclear compartment, the nucleolus. In cells that are actively transcribing the rDNA, nucleoli appear to be composed of three compartments: the fibrillar center, which contains DNA that is not being transcribed; the dense fibrillar component, where rDNA transcription occurs; and the peripheral granular component, where pre-rRNA processing and pre-ribosome assembly take place (1, 2). In proliferating cells, RNA polymerase I (pol I)1 and other components of the transcription complex localize to the dense fibrillar component, whereas molecules essential for rRNA processing, like fibrillarin and the small nucleolar RNAs, localize to the peripheral granular component (for review, see Refs. 3–5). The rate at which the rDNA is transcribed in actively dividing cells is remarkable. Electron microscopic analysis shows that during active rDNA transcription in metazoan cells, the spacing between pol I complexes is only 100 base pairs (6).

One of the most abundant proteins in the nucleoli of vertebrate cells is the highly conserved protein, nucleolin. Mammalian nucleolin is 709 amino acids in length and consists of an unusual grouping of sequence and structural motifs (7–14). The N-terminal region of nucleolin houses several long stretches of acidic residues with the potential to function as “acid blobs” in activation of transcription (15). The central region of nucleolin contains four RNA binding domains (RBDDs; also called RNA recognition motifs or RRMs). RBDDs are common among proteins that interact with single-stranded nucleic acids (16, 17), and the RBDDs of nucleolin are believed to mediate interactions of nucleolin with RNA (18–22). The C terminus of nucleolin contains nine repeats of the tripeptide motif arginine-glycine-glycine (RGG), in which the arginine residues are dimethylated (23, 24).

The distribution of nucleolin within the nucleolus is unusual. Whereas proteins like pol I and fibrillarin appear to be restricted to a single compartment of the nucleolus, nucleolin is abundant within both the dense fibrillar component and the granular component (for review, see Ref. 4). The presence of nucleolin in the peripheral granular component is consistent with the participation of nucleolin in rRNA processing and ribosome assembly (19–22). The fact that nucleolin is abundant within the dense fibrillar component suggests that nucleolin also functions in other processes, including transcription, replication, or recombination of the rDNA. Nonetheless, conserved and specific interactions of nucleolin with the duplex rDNA have not been reported.

The rDNA transcription unit includes the regions that template mature 18, 5.8, and 28 S RNAs and external and internal transcribed spacer regions (Fig. 1A). In all eukaryotes, the entire transcribed region of the rDNA is very rich in the base guanine (34.2% in humans) within the spacers, as well as within the regions that template the mature RNAs. The G-richness is restricted to a single strand, the non-template strand, and most guanines are within runs that contain three or more consecutive Gs (Fig. 1B).

Single-stranded DNAs that contain runs of three or more consecutive guanine residues readily self-associate in vitro to form structures stabilized by G-G pairing (25–31). In these structures, guanines interact via Hoogsteen bonding to form planar rings called G quartets (Fig. 2A), and the G quartets stack upon each other to stabilize higher order structures (Fig. 2B). That guanine-guanine interactions could occur readily in solution was first established nearly 40 years ago (32). Although G-G-paired DNA has not been directly observed in vivo, G-G-paired structures form rapidly and spontaneously in vitro and are very stable once formed. Because of its sequence, the G-rich strand of the rDNA has considerable potential to form G-G-paired structures (Fig. 2A). Formation of such structures may be stimulated by the unwinding and localized denaturation that accompanies rDNA transcription.
separable domains of nucleolin can bind G4 DNA, one protein with nucleic acid. Mutational analysis shows that two sentences a remarkably high affinity for interaction of a eukaryotic right Hoogsteen bonding. shows four guanine residues forming a planar array stabilized by left tide.

Interaction of Nucleolin with G-G-paired rDNA

The observations presented above have led us to investigate the interaction of nucleolin with G-G-paired DNA. Here we report that mammalian nucleolin binds tightly and specifically to both four-stranded G4 DNA and two-stranded G2 DNA. The dissociation constant for binding is $K_d = 1$ nM, which represents a remarkably high affinity for interaction of a eukaryotic protein with nucleic acid. Mutational analysis shows that two separable domains of nucleolin can bind G4 DNA, one comprised of the four RBDs (RBD-1,2,3,4) and the other comprised of the C-terminal Arg-Gly-Gly repeats (RGG$_9$). These results suggest that G-G-paired DNA is a natural binding target of nucleolin within the nucleolus. Nucleolin may, therefore, be an architectural factor that functions to organize the G-rich non-template strand of the rDNA during transcription, replication, or recombination.

**Experimental Procedures**

**Plasmid Construction**—The backbone for construction of deletion mutants of nucleolin was the plasmid pNuc-1,2,3,4-RGG$_9$, which carries human nucleolin residues 284–709, including all four RBDs and the nine RGG$_9$s, fused at the N terminus to *Escherichia coli* maltose-binding protein. Construction of pNuc-1,2,3,4-RGG$_9$ has been described previously (Ref. 14, where it was referred to as pMalNuc). Mutants were constructed as follows. For pNuc-1,2-RGG$_9$, RBDs 1 and 2 (nucleolin residues 268–470) were polymerase chain reaction-amplified from the pNuc-1,2,3,4-RGG$_9$ template with deoxyoligonucleotides 26937 (GGCGATCCAAAGGACGTCCTGAAAGC) and 26938 (GACACCTTGG-ATTGACCATCTTTCTCCT) and ligated to pNuc-1,2,3,4-RGG$_9$ cleaved with these enzymes. For pNuc-3,4-RGG$_9$, the same procedure was used as for pNuc-1,2-RGG$_9$, except that the polymerase chain reaction was carried out with deoxyoligonucleotides 26939 (GGGAGTTCAATAGGACCTGAGTG) and 26940 (TGGTTCACCCCTTAGGTTTGGG) to amplify RBDs 3 and 4 (nucleolin residues 478–647). For pNuc-1,2,3,4-RGG$_9$, pNuc-1,2,3,4-RGG$_9$ was digested with BamHI and EcoRI, which excised the GGGs, and a synthetic linker made by annealing deoxyoligonucleotides TAACTAATGGCTGTGA and TCAAGCTTCTTT was inserted to provide a stop codon. For pNuc-3,4-RGG$_9$, pNuc-1,2,3,4-RGG$_9$ was digested with BamHI and BsuRI, the 5’-overhang was filled with Klenow, and a 94-base pair fragment was liberated by HindIII digestion and inserted into pNuc-1,2,3,4-RGG$_9$ that had been digested with BamHI and 3′-filled. All clones involving polymerase chain reaction amplification were sequenced throughout the amplified region.

**Protein Purification**—Full-length (106-kDa) murine nucleolin was purified starting with nuclear extract prepared from PD31 pre-B cells and chromatographed on heparin-agarose resin as described (33). Fractions containing nucleolin were identified at this and subsequent steps by blotting with anti-nucleolin antibodies (14). Fractions were dialyzed against Buffer L (10 mM Tris, pH 7.4, and 1 mM EDTA) containing 0.2 mM NaCl, 0.1 mM dithiorthreitol, and 0.1 mM phenylmethylsulfonyl fluoride, applied to a Hi-Trap Q (Amersham Pharmacia Biotech) column, and eluted with a 0.2–1.0 M NaCl linear gradient in Buffer L. Fractions containing nucleolin were dialyzed against Buffer B (10 mM Hepes, pH 7.5, 1 mM EDTA) containing 0.2–1.0 M NaCl gradient in Buffer L. Fractions containing nucleolin were dialyzed against Buffer B (10 mM Hepes, pH 7.5, 1 mM EDTA) containing 0.2–1.0 M NaCl gradient in Buffer L. Nucleolin-containing fractions were dialyzed against Buffer L containing 0.1 M NaCl, applied to polygaunosine-agarose resin (Sigma), and eluted with Buffer L containing 1.0 M NaCl.

All recombinant proteins were produced by overexpression as described previously (14). As the final step in purification, fusion proteins that contained RGG$_9$s domains were applied to Mono S and eluted with a 0.05–1.0 M NaCl linear gradient in Buffer B. Other proteins were fractionated instead by Mono Q chromatography and eluted with a 0.05–1.0 M NaCl linear gradient in Buffer L. All purified fusion proteins were chromatographed as single species on SDS-polyacrylamide gel electrophoresis. Concentrations of proteins were determined by Bradford microcylometry (Bio-Rad).

**Formation of G-G-paired DNAs**—Sequences of oligonucleotides used in binding analyses were: ETS-1, TCTCTCGGCTGGCCGGGGGCTGTC-GGGTTTGGGCTCGCC; ETS-2, GAGCTGATGCA and TCAGCTACTTAC was inserted to provide a stop codon. For pNuc-RGG$_9$, pNuc-1,2,3,4-RGG$_9$ was digested with HI and BsuHI and ligated to pNuc-1,2,3,4-RGG$_9$ cleaved with these enzymes. For pNuc-3,4-RGG$_9$, the same procedure was used as for pNuc-1,2-RGG$_9$, except that the polymerase chain reaction was carried out with deoxyoligonucleotides 26939 (GGGAGTTCAATAGGACCTGAGTG) and 26940 (TGGTTCACCCCTTAGGTTTGGG) to amplify RBDs 3 and 4 (nucleolin residues 478–647). For pNuc-1,2,3,4-RGG$_9$, pNuc-1,2,3,4-RGG$_9$ was digested with BamHI and EcoRI, which excised the GGGs, and a synthetic linker made by annealing deoxyoligonucleotides TAACTAATGGCTGTGA and TCAAGCTTCTTT was inserted to provide a stop codon. For pNuc-3,4-RGG$_9$, pNuc-1,2,3,4-RGG$_9$ was digested with BamHI and BsuRI, the 5’-overhang was filled with Klenow, and a 94-base pair fragment was liberated by HindIII digestion and inserted into pNuc-1,2,3,4-RGG$_9$ that had been digested with BamHI and 3′-filled. All clones involving polymerase chain reaction amplification were sequenced throughout the amplified region.

**DNA Mobility Shift Analysis and Measurements of Binding Affinities**—Binding to G4 DNA and G2 DNA was carried out in 15-mM reac-
Assay of murine nucleolin binding to G4 DNA formed from 32P-labeled phorImager analysis of the dried gels, and estimated by gel mobility shift assays in which binding to a fixed protein was performed at three DNA concentrations, 330 fM, 3.3 pM, and 33 pM; Arrows identify protein-DNA complexes of two distinct mobilities; free indicates unbound DNA.

tions containing 10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 100 μg/ml bovine serum albumin, and 1 fmol of 32P-labeled DNA for 30 min at 37 °C, glycerol was added to a final concentration of 5% (w/v), and complexes were resolved by gel electrophoresis on 6% (29:1, acrylamide: bisacrylamide) 0.5 × TBE gels at 5 V/cm for 10 h at 4 °C. Affinities were estimated by gel mobility shift assays in which binding to a fixed amount of G4 DNA was assayed in the presence of increasing amounts of protein. Protein-DNA complex formation was quantitated by PhosphorImager analysis of the dried gels, and $K_D$ values were calculated by plotting the fraction of bound DNA at each protein concentration. Reported $K_D$ values are averages from at least three separate experiments. To verify the very low $K_D$ values for G4 DNA interactions, assays were performed at three DNA concentrations, 330 fm, 3.3 pm, and 33 pm; the apparent $K_D$ was the same at all concentrations.

RESULTS

Binding of G4 DNA by Endogenous Nucleolin—G4 DNA forms spontaneously in solutions of G-rich synthetic oligonucleotides, but to form G4 DNA at very high yield, synthetic oligonucleotides were incubated at high concentrations at 60 °C for 48 h (32). Under these conditions, over 90% of the starting material typically formed G4 DNA. G4 DNA formation was verified in all cases by dimethyl sulfate footprinting (35). Fig. 2C shows a typical footprint obtained by probing G4 DNA formed from the ETS-1 oligonucleotide. This 40-mer derives from a sequence in the 5′-ETS region of the human rDNA and represents one of many regions in the rDNA that will readily form G-G-paired structures in vitro. Structures of other G-G-paired DNAs used in binding assays were similarly verified (data not shown).

Nucleolin was purified from nuclear extracts of murine PD31 pre-B cells. The protein preparation was shown to be homogeneous by silver staining, and the identification of the 106-kDa protein as nucleolin was confirmed by Western blot analysis with anti-nucleolin antibodies (Fig. 3A). The ability of nucleolin to bind G4 DNA was assayed by gel mobility shift using G4 DNA formed from the ETS-1 oligonucleotide. Binding analysis showed that full-length mammalian nucleolin binds very tightly to G4 DNA formed from the ETS-1 oligonucleotide: $K_D = 1 \text{ nM}$ (Fig. 3B). Similar results were obtained with G4 DNA generated from other oligonucleotides (not shown).

Binding of G4 DNA by Recombinant Nucleolin—Full-length nucleolin cannot be expressed in E. coli, but deletion of the N terminus permits good expression of recombinant protein (14). The Nuc-1,2,3,4-RGGg fusion protein (nucleolin residues 284–709), which carries RBDs 1, 2, 3, and 4 and the RGGg domain, was assayed for interaction with G4 DNA formed from the ETS-1 oligonucleotide and shown to bind this G4 DNA with $K_D = 0.5 \text{ nM}$ (Fig. 4A). Binding produced two shifted complexes of distinct mobilities, which probably represent interaction of more than one polypeptide with each G4 DNA substrate, via protein-DNA or protein-protein interactions. Nuc-1,2,3,4-RGGg bound comparably with G4 DNA formed from the ETS-1 oligonucleotide and other oligonucleotides (data not shown). Incubation of G4 DNA with nucleolin did not permanently alter DNA structure, because following addition of SDS and proteinase K to the binding reaction, all DNA migrated as free G4 DNA (not shown). MBP did not bind G4 DNA ($K_D > 40 \text{ nM}$) (Fig. 4B).

Both full-length mammalian nucleolin and recombinant nucleolin (residues 284–709) therefore bind G4 DNA with high affinity. Nucleolin undergoes extensive posttranslational modifications, including phosphorylation and dimethylargininylation (36–38). The high affinity binding of recombinant nucleolin shows that these modifications are not essential for interaction with G-G-paired DNA.

The Nucleolin RBD-1,2,3,4 Domain Binds G4 DNA and G2 DNA—To identify the domains of nucleolin that interact with G4 DNA, we began by separating the domain comprised of the four RBDs from the C-terminal RGGg domain. We assayed binding to G4 DNA by recombinant Nuc-1,2,3,4, which carries RBDs 1, 2, 3, and 4. Nuc-1,2,3,4 bound to G4 DNA (Fig. 5). The dissociation constant for this interaction ($K_D = 0.5 \text{ nM}$) is comparable to that of Nuc-1,2,3,4-RGGg binding to G4 DNA (Fig. 4). G-rich DNAs can form several different structures, including G4 DNA, in which G-G pairing stabilizes interactions between four parallel strands, and G2 DNA, in which two strands associate in antiparallel orientation (for review, see Ref. 39; see also Fig. 1B). Nuc-1,2,3,4 bound to G2 DNA with affinity similar to, but slightly lower than, that observed in assays of G4 DNA binding (Fig. 5).

RGGg Binds G4 DNA—The 41-amino acid C-terminal region of nucleolin is comprised of nine repeats of the motif RGG. Nuc-RGGg, which expresses the RGGg domain as a chimeric MBP-fusion protein, bound G4 DNA with $K_D = 3.3 \text{ nM}$ (Fig. 6A). The RGGg domain, therefore, comprises a second and independent high affinity G4 DNA binding domain. Competition experiments carried out in the presence of cold competitor G4 DNA or single-stranded DNA showed that G4 DNA effectively competed for binding, whereas the single-stranded oligonucleotide had no effect, even at 1000-fold molar excess (Fig. 6B). Additional binding and competition studies demonstrated that recombinant Nuc-RGGg does not bind duplex DNA or
single-stranded DNA ($K_D > 1 \mu M$; data not shown) and that deletion of five of the nine RGG repeats (Nuc-RGG4) abolished G4 DNA interaction (Fig. 7). The RGG9 domain of nucleolin binds comparably to G4 DNAs formed from other synthetic oligonucleotides and, thus, appears to recognize G4 DNA structures independent of sequence context.

**RBD-3,4 Combines with RGG9 to Produce a High Affinity G4 DNA Binding Domain**—Having identified RBD-1,2,3,4 and RGG9 as separable G4 DNA binding domains, additional deletion analysis was carried out in an attempt to define smaller subdomains capable of high affinity interaction with G4 DNA. Binding assays were carried out with eight different deletion mutants, expressed in _E. coli_ as chimeric MBP fusion proteins, and purified to homogeneity. Results of these experiments, summarized in Fig. 7, showed that Nuc-3,4-RGG9, which carried RBDs 3 and 4 and the RGG9 domain, bound G4 DNA with high affinity ($K_D = 0.5 \text{ nM}$). Binding affinity was decreased 4-fold ($K_D = 2 \text{ nM}$) when RBDs 1 and 2 were substituted for RBDs 3 and 4 to produce Nuc-1,2-RGG9.

The importance of the RGG9 domain in G4 DNA recognition is reinforced by the observation that whereas the Nuc-3,4-RGG9 chimera-bound G4 DNA with relatively high affinity, deletion of RGG9 to produce Nuc-3,4 resulted in a complete loss of binding ($K_D > 40 \text{ nM}$). Similarly, Nuc-1,2 was not active in G4 DNA binding ($K_D > 40 \text{ nM}$). Finally, complete loss of G4 DNA binding occurred when the RGG9 region was truncated by deletion of the N-terminal five RGG repeats to create MBP-RGG4 (Fig. 7).

**DISCUSSION**

We have shown that the abundant nucleolar protein, nucleolin, binds G-G-paired DNA with very high affinity ($K_D = 1 \text{ nM}$). Nucleolin can bind to both four-stranded G4 DNA and two-stranded G2 DNA, and nucleolin recognizes G-G-paired structures independent of sequence context. The remarkably high binding affinities suggest that G-G-paired structures are binding targets of nucleolin _in vivo_. The observation that nucleolin binds G-G-paired structures independent of sequence context shows that this protein will be able to bind G-G-paired structures wherever they might form within the G-rich rDNA.

**Dynamic Formation of G-G-paired DNA in the Nucleolus**—Most nuclear DNA is double-stranded, and complementary base pairing will normally protect duplex DNA from forming G-G-paired structures. However, duplex DNA becomes transiently single-stranded during three critical and dynamic processes: transcription, replication, and recombination. Cells have developed sophisticated mechanisms to prevent DNA from adopting alternative structures, including a variety of proteins that bind to transiently exposed single-stranded regions. Nonetheless, these mechanisms are not foolproof. For example, there is considerable evidence that triplet repeat expansion results from formation of non-Watson-Crick structures during replication (see Ref. 40 and references therein).

The sequence composition and the strand asymmetry of the rDNA provide it with considerable potential to form G-G-paired structures. The rDNA is G-rich on the top (non-template) strand, not only within the region transcribed into pre-rRNA but also within the spacers (Fig. 1). During active transcription, pol I molecules pack at extremely high density on the rDNA repeats; electron micrographic analysis shows that the spacing between pol I complexes is only 100 base pairs (6). Transcription at this level requires that a considerable fraction of the rDNA duplex be denatured. We hypothesize that G-G-paired structures form within the G-rich top strand of the rDNA during transcription or when the duplex is transiently denatured during replication or recombination. G-G-paired structures are very stable once formed (26) and would not be predicted to dissociate spontaneously _in vivo_.

Other experiments provide further support for the notion of a dynamic process of formation and unwinding of G-G-paired structures within the active rDNA. We have recently shown that G-G-paired DNA is the preferred substrate of two eukaryotic helicases, the human BLM helicase, which is deficient in Bloom’s syndrome (41), and the _Saccharomyces cerevisiae_ Sgs1p helicase (42). Both these helicases are members of the highly conserved RecQ helicase family. Moreover, _S. cerevisiae_
Sgs1p localizes predominantly to the nucleolus (43, 44), where it could function to maintain the structure of the G-rich rDNA. The human functional homolog of Sgs1p in S. cerevisiae appears to be the WRN helicase (deficient in Werner’s syndrome). Like Sgs1p, WRN is a RecQ family helicase that is predominantly nucleolar in localization (45, 46). Unwinding activity mapped to the conserved helicase core domain of Sgs1p (42), strongly suggesting that preferential activity on G-G-paired substrates may be a general property of helicases in this family. It is therefore very likely that WRN will also prove to be active on G-paired rDNA substrates.

Nucleolin as an Architectural Factor in rDNA Transcription, Replication, or Recombination—Two separable domains within nucleolin can bind G-paired structures, one comprised of the RBDs 1, 2, 3, and 4 and the other comprised of the C-terminal RGG three domain. The presence of two independent G-G DNA binding domains would contribute to the ability of nucleolin to organize G-paired regions. Nucleolin may thus be an architectural factor, in effect forming a scaffolding for the structured G-rich strand. The presence of long acidic runs in the N terminus of nucleolin is consistent with its function in transcription, but nucleolin is a complex molecule with multiple distinct domains, and it may have multiple functions. We have identified nucleolin as one component of a heterodimeric protein, LR1, induced specifically in B cells activated for immunoglobulin heavy chain switch recombination (14, 33, 47). The rDNA localization (48–50), whereas the N-terminal acidic region is dispensable. The ability to interact with G-G-paired nucleic acids may, therefore, be essential to localization or retention of nucleolin within the nucleolus.

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REFERENCES
1. Puvion-Dutilleul, F., Bachellerie, J.-P., and Puvion, E. (1991) Chromosoma 100, 395–409
2. Puvion-Dutilleul, F., Puvion, E., and Bachellerie, J.-P. (1997) Chromosoma 105, 496–505
3. Fakan, S., and Puvion, E. (1980) Int. Rev. Cytol. 65, 225–299
4. Jordan, G. (1987) Nature 329, 489–490
5. Gerbi, S. A. (1995) Biochem. Cell Biol. 73, 845–858
6. Osheim, Y., Mougey, E. B., Windle, J., Anderson, M., O’Reilly, M., Miller, O. L., Dreyer, A., and Sollner-Webb, E. (1996) J. Cell Biol. 135, 943–954
7. Lapeyre, B., Bourbon, H., and Amalric, F. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1472–1476
8. Bourbon, H.-M., Lapeyre, B., and Amalric, F. (1988) J. Mol. Biol. 200, 627–638
9. Bourbon, H.-M., and Amalric, F. (1990) Gene 88, 157–166
10. Srivastava, M., McBride, O. W., Fleming, P. J., Pollard, H. B., and Burns, A. L. (1990) J. Biol. Chem. 265, 14922–14931
11. Srivastava, M., Fleming, P. J., Pollard, H., and Burns, A. L. (1989) FEBS Lett. 230, 99–105
12. Maridor, G., and Nigg, E. A. (1990) Nucleic Acids Res. 18, 1286
13. Rankin, M. L., Heine, M. A., Xiao, S., LeBlanc, M. D., Nelson, J. W., and DiMari, P. J. (1995) Nucleic Acids Res. 23, 169
14. Hanakahi, L. A., Dempsey, L. A., Li, M.-J., and Maizels, N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3605–3610
15. Ptashne, M. (1988) Nature 333, 683–689
16. Kenan, D. J.,_query, C. C., and Reene, J. D. (1991) Trends Biochem. Sci. 16, 214–220
17. Birsey, E., Kumar, S., and Krainer, A. R. (1993) Nucleic Acids Res. 21, 5803–5816
18. Ghisolfi-Nieto, L., Joseph, G., Puvion-Dutilleul, F., Amalric, F., and Bouvet, F. (1996) J. Mol. Biol. 260, 34–53
19. Ginisty, H., Amalric, F., and Bouvet, P. (1998) EMBO J. 17, 1476–1486
20. Seun, G., Joseph, G., Ghisolfi, L., Buxan, M., Erard, M., Amalric, F., and Bouvet, P. (1997) J. Biol. Chem. 272, 13109–13116
21. Bouvet, P., Jain, C., Belasco, J. B., Amalric, F., and Erard, M. (1997) EMBO J. 16, 5235–5246
22. Bouvet, P., Diaz, J.-J., Kindbeiter, K., Madjar, J.-J., and Amalric, F. (1998) J. Biol. Chem. 273, 19025–19029
23. Lischew, M. A., Cook, R. G., Ahn, Y. S., Yeoman, L. C., and Busch, H. (1985) Biochemistry 24, 6025–6028
24. Lapeyre, B., Amalric, F., Ghaffari, S. H., Venkatakrupa Rao, S. V., Dumbar, T. S., and Olson, M. J. O. (1986) J. Biol. Chem. 261, 9167–9173
25. Sen, D., and Gilbert, W. (1988) Nature 334, 364–366
26. Sen, D., and Gilbert, W. (1990) Nature 344, 410–414
27. Williamson, J. R., Raghuraman, M. K., and Cech, T. R. (1989) Cell 59, 871–880
28. Kang, C.-H., Zhang, X., Ratliff, R., Moyeris, R., and Rich, A. (1992) Nature 356, 126–131
29. Kim, J., Cheong, C., and Moore, P. B. (1991) Nature 351, 331–332
30. Wang, Y., and Patel, D. J. (1992) Biochemistry 31, 812–819
31. Laughan, G., Murchie, A. I., Norman, D. G., Moore, M. H., Moody, P. C., Liley, D. M., and Lui, B. (1984) Science 225, 520–524
32. Gellert, M., Lipsett, M. N., and Davies, D. R. (1986) Proc. Natl. Acad. Sci. U. S. A. 84, 2014–2018
33. Dempsey, L. A., Hanakahi, L. A., and Maizels, N. (1998) J. Biol. Chem. 273, 12294–12299
34. Sen, D., and Gilbert, W. (1992) Biochemistry 31, 65–70
35. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499–560
36. Belenguer, P., Caizergues-Ferrer, M., Belenguer, P., Lapeyre, B., Amalric, F., Wallace, M. O., and Oleo, M. O. J. (1987) Biocemistry 26, 7876–7883
37. Belenguer, P., Baldin, V., Mathieu, C., Prats, H., Bensaid, M., Bouché, G., and Amalric, F. (1989) Nucleic Acids Res. 17, 6625–6636
38. Belenguer, P., Caizergues-Ferrer, M., Labbé, J., Doree, M., and Amalric, F. (1990) Mol. Cell. Biol. 10, 3607–3618
39. Williamson, J. R. (1994) Annu. Rev. Biophys. Biomol. Struct. 23, 703–730
40. Gacy, A. M., Goellner, G. M., Sipios, C., Chen, X., Gupta, G., Bradbury, E. M., Dyer, R. B., Mikelse, M., and Olson, M. O. J. (1996) J. Mol. Biol. 259, 1239–1250
41. Heine, M. A., Rankin, M. L., and DiMario, P. J. (1995) Mol. Cell. Biol. 4, 1239–1250
42. Schmidt-Zachermann, M. S., and Nigg, E. A. (1993) J. Cell Biol. 105, 799–806
43. Créancier, L., Prats, H., Zanibellato, C., Amalric, F., and Bugler, B. (1995) Mol. Cell. Biol. 15, 7257–7259