FEBS Letters 352 (1994) 380–384

FEBS 14608

Effect of unmodified triple helix-forming oligodeoxyribonucleotide targeted to human multidrug-resistance gene *mdr1* in MDR cancer cells

Bruna Scaggiante[a], Carla Morassutti[a], Giuseppe Tolazzi[a], Angela Michelutti[b], Michele Baccarani[b], Franco Quadrifoglio[a,*]

[a]*Department of Biomedical Sciences and Technologies, via Gervasutta 48, University of Udine, I-33100 Udine, Italy*
[b]*Department of Morphological and Clinical Research, piazzale Kolbe 2, University of Udine, I-33100 Udine, Italy*

Received 17 August 1994

Abstract The human *mdr1* gene encodes a transmembrane glycoprotein the over-expression of which is associated with development of multidrug resistance in human tumor cells. A negative modulation of human *mdr1* has been attempted via a 27-mer unmodified triple helix-forming oligonucleotide, named 1D, targeted to a homopurine sequence in the coding region of the gene. By administering 10 μM of 1D we could find a significant reduction in MDR1 mRNA levels in the human drug-resistant cell line CEM-VLB100. This effect appears to be specific and due to a transient block of RNA polymerase mediated by triple helix formation.

Key words: Triple helix; *mdr1*; Multidrug resistance; Oligodeoxyribonucleotide; CEM-VLB100 cell

1. Introduction

Studies on nucleic acid triple helices date back more than 30 years [1], but only recently has interest in triple helix DNA structures increased they represent potential biological reagents for chromosome mapping and gene analysis [2]. These unusual structures are formed by the interaction of a homopyr/homopur sequence with the major groove of a homopur:homopyr Watson-Crick (W-C) target via Hoogsteen or 'reverse' Hoogsteen hydrogen bonds. Essentially, three major triple helix structural motives can be recognized: the acidic pH-dependent pyrimidine one based on C^+-GC. T-AT triplets, in which the third strand is parallel to the homopurinic W-C sequence; the pH-independent purine and mixed purine/pyrimidine ones based on G-GC, A-AT and G-GC, T-AT triplets, respectively, in which the third strand is orientated antiparallel or parallel with respect to the homopurinic W-C sequence depending on its base composition [3]. The potential selective interaction within megabase DNA has suggested the possibility of exogenously modulating gene expression by intermolecular triplex formation [5,6]. Many in vitro experiments have demonstrated that triple helix-forming oligodeoxyribonucleotides (ODNs) can selectively inhibit gene transcription, either by preventing transcription factor binding to promoters [6,7] or by blocking transcription elongation [8,9]. This has led to consideration of the triple helix 'anti-gene' strategy as a new therapeutic approach to selectively suppress unwanted gene expression in vivo. The apparent limitation of triplex targeting in vivo has been overcome following evidence that relatively long stretches of homopurine:homopyrimidine sequences are often present within the eukaryotic genome [10] and that their frequency increases 3.5–4 times in higher eukaryotes [11]. In human cells, transcription inhibition at the genomic level by purinic triple helix-forming ODNs has been described for c-*myc* [12] and IL2Rα [13] genes.

The intrinsic or acquired pleiotropic resistance to drugs, called multidrug-resistance (MDR), is one of the major obstacles in cancer treatment [14]. In human tumors, the MDR phenomenon is often associated with the over-expression, and sometimes the amplification [15–17] of the *mdr1* gene encoding a constitutive transmembrane glycoprotein (P-gp). Although the precise physiological function of P-gp is still unknown, in cancer-resistant cells it is responsible for a rapid energy-dependent efflux of many structurally and funcionally unrelated anti-tumor chemicals [18]. Current strategies to circumvent drug resistance involve the administration of toxic agents, which have adverse side effects [14]. Although at the initial stage, studies in mice seem to have demonstrated that administration of ODNs can reach therapeutic values in organs and can be well tolerated [19–21].

We tried to inhibit human *mdr1* gene expression in MDR cells by intermolecular triple helix formation and demonstrate that a natural, unmodified triple helix-forming ODN can significantly reduce MDR1 mRNA levels in a MDR cell line.

2. Materials and methods

2.1. Oligonucleotide synthesis and purification

ODNs or modified ODNs were synthesized on an automated DNA synthesizer Model 380 B (Applied Biosystem) by the phosphoramidite method, according to standard procedures (1 μmol scale), and purified by FPLC on an anionic-exchange MONO Q HR 5/5 column (Pharmacia) utilizing an ammonium bicarbonate gradient. The purity of ODNs was controlled on a 20% acrylamide/7 M urea gel under denaturing conditions.

2.2. Triple helix detection in vitro

Optical melting curves were performed at 260 nm on a Cary spectrophotometer model 220 (Varian) equipped with a Tandy 102 temperature programmer, connected to a Haake K.F3 thermostat. Equimolar concentrations of ODN and its corresponding synthetic target duplex were mixed in 75 mM Tris-HCl, pH 7.2, containing 75 mM NaCl, 10 mM $MgCl_2$, 2 mM spermine, and heated at a rate of 0.4°C/min from 5 to 80°C.

Triplex gel-retardation assays were performed with 5′ ^{32}P-labeled

*Corresponding author: Fax: (39) (432) 600 828.

Abbreviations: W-C, Watson and Crick; ODN, oligodeoxyribonucleotide.

SSDI 0014-5793(94)00995-3

ODN prepared by standard procedures with T4 polynucleotide kinase (Promega). ODN and ODN annealed with its ^{32}P-labelled duplex (ODN/duplex, 100/1) in 75 mM Tris-HCl, pH 7.2, containing 10 mM $MgCl_2$, 75 mM NaCl and 2 mM spermine, were loaded on 15% acrylamide non-denaturing gels and electrophoresis performed at 20°C for 2 h, utilizing 0.1 M sodium phosphate buffer, pH 7.2, at 6 V/cm. Triple helix formation was identified as a band migrating slower than that of the duplex.

2.3. Cell cultures and ODN administration

A human MDR cell line of leukemic T-lymphoblasts (CEM-VLB100) [22] was cultured in RPMI 1640 containing 10% fetal calf serum, 2 mM L-Gln, 100 U/ml penicillin and 100 μg/ml streptomycin (Seromed; Biochrom KG). The MDR phenotype was mantained with 50 ng/ml vinblastine. Prior to administering ODN, the cells were cultured in drug-free medium for 24 h. For RNA quantitation experiments 5×10^6 cells were seeded in complete medium and one dose of ODNs, ranging from 2.5 to 10 μM, were supplied in a final volume of 10 ml.

To quantify cell ODN uptake, 2×10^5 cells/ml were seeded in 96-well microtiter plates and incubated at 37°C with 15 μM ODN containing trace amounts of 5′ ^{32}P-labeled ODN purified by Sephadex G25 exclusion chromatography to remove unincorporated label. The medium was then discarded, the cells rinsed twice with PBS, and lysed with 50 μl of HIRT solution (10 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.6% SDS). The total radioactivity was evaluated as scintillation counts (β-Counter LS 1801; Beckman).

To detect the presence of undegraded ODN in living cells, 0.5×10^6 CEM-VLB100 cells were cultured in 1 ml of complete medium. The cells were exposed to 20 μM ODN containing trace amounts of 5′ ^{32}P-labeled ODN at 37°C for variable times. The cells were collected by centrifugation at 400 × g for 5 min, rinsed once with 0.2 M Gly/NaOH, pH 4.0, twice with physiological saline solution, and then disrupted with 0.2% SDS/4 M urea. The samples were then loaded onto 15% acrylamide/7 M urea gels, electrophoresed for 2 h at 8 V/cm, 42°C, and the gels then exposed to Kodak XAR-OMAT film.

2.4. Total RNA extraction and quantitation

Total RNA was extracted from the cells as described by Chomczynsky and Sacchi [23]. The RNA quality was evaluated by ribosomal band integrity in 1% agarose/formaldehyde gels. From 10 to 20 μg of total RNA were transferred on to nylon filters (Magna charge, MSI) with 10 × SSC (1.5 M NaCl, 0.15 M sodium citrate, pH 7.0). Northern blot filters were hybridized with pDHR5A MDR1 cDNA [24] at 65°C in Church solution (0.5 M sodium phosphate buffer, pH 7.2, containing 7% SDS and 1 mM EDTA) and specific probe recognition was assured by high stringency conditions of temperature and rinsing (from 5 × to 0.1 × SSC, 65°C). The same filters were then rehybridized with human GPDH or mouse β-actin cDNA probes at 58°C, rinsing the filters from 5 × to 1 × SSC at the same temperature. The filters were exposed to Kodak XAR-OMAT films. All probes were labeled with [α-^{32}P]dCTP by the random hexamer priming method. The quality of detection of MDR1 mRNA was assured by the absence of signals in the line corresponding to sensitive non-MDR CCRF-CEM cells (data not shown).

Autoradiogram band intensities were quantified by densitometric laser scanning (Ultroscan XL, Pharmacia). Laser scan densitometric values of MDR1 mRNA of treated cells were normalized to that of control GPDH mRNA and expressed as % difference with respect to MDR1/GPDH normalized values of untreated or control ODN-treated samples. Comparison of mRNA ratios were made only between samples on the same filter and which had been probed under identical conditions.

3. Results and discussion

Within the genomic sequence of the human *mdr1* gene [25] we identified a homopurinic stretch in exon 3 of the coding region from position 69 to 93. We synthesized a natural phosphodiester 27-mer ODN, named 1D, orientated parallel with respect to the homopurine W-C strand, and its antiparallel sequence, named 1E. The ODNs were also designed to recognize the two last conserved bases of intron 2 and to establish

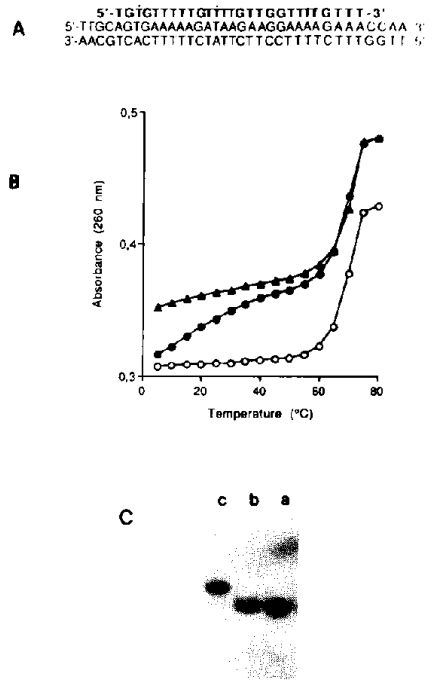

Fig. 1. (A) Sequence of the putative *mdr1* and the triplex-forming region of ODN 1D. Bases in the 1D sequence indicated with arrowheads correspond to mismatch sites. (B) In vitro triple helix formation demonstrated by thermal denaturation curves: 4 nmol of the 35 bp synthetic target strands in 2 ml were annealed alone or with equimolar concentrations of 1D or 1E (antiparallel with respect to 1D) as described in section 2. The optical values obtained at 260 nm for the duplex and the triplex are plotted as a function of temperature (○, duplex alone; ●, duplex + 1D; ▲, duplex + 1E). (C) Triplex gel-retardation assay. 0.1 μM of synthetic target, containing trace amounts of labeled duplex, was annealed with 10 μM of 1D or 1E (antiparallel with respect to 1D) as described in section 2. All samples were heated at 90°C and then allowed to cool at room temperature for at least 2 h prior to their loading into a non-denaturing gel (a, duplex; b, duplex + 1E; c, duplex + 1D).

T-AT and G-GC triplets (Fig. 1A). In thermal denaturation experiments performed at pH 7.2, 1D in the presence of the corresponding 35 bp synthetic duplex at equimolar concentrations was able to produce a biphasic profile, characteristic of third strand dissociation from a W-C duplex (Fig. 1B). In fact, since the triple helix has a lower stability than the corresponding duplex, in thermal denaturation experiments the dissociation of the triplex precedes duplex melting so that a biphasic curve results [26,27]. In contrast, addition of the antiparallel sequence 1E to the W-C duplex did not result in a triple helix dissociation curve, but only in an increase in W-C melting optical values due to the absorbance contribution of the single strand. Thus, as expected, the preferred orientation of the third strand is parallel with respect to the homopurinic W-C strand, according to its prevalent pyrimidinic composition [28]. Moreover, although 1D showed slow kinetics of association and partial stability in a triplex form at 37°C, it was able to produce a retardation in the gel electrophoresis migration of the duplex, whereas 1E did not significantly change duplex migration (Fig. 1C).

These data suggested that 1D might possess sufficient duplex sequence selectivity under physiological conditions to be a can-

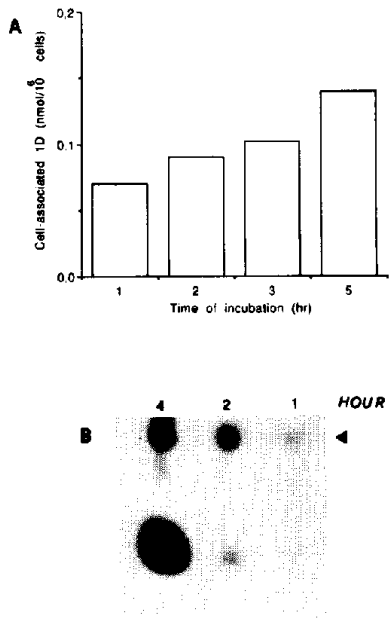

Fig. 2. (A) Cell ODN uptake. 2×10^4 CEM-VLB100 cells were incubated at 37°C for the indicated times with 15 μM 1D containing trace amounts of labeled strand. Total radioactivity of whole-cell lysates was evaluated by scintillation counts. The specific activity of the culture medium was determined for each sample and did not change over the course of the experiments (1,800,000 cpm/well). (B) Recovery of full-length ODN from the cells. 0.5×10^6 CEM-VLB100 cells were incubated at 37°C for the indicated times with 20 μM 1D containing trace amounts of labeled strand. Cellular lysate was loaded on a 15% acrylamide denaturing gel. Labeled 1D was loaded under the same conditions to mark undegraded ODN migration (arrowhead).

didate for inhibition of *mdr1* gene transcription in human MDR cell lines.

The cell uptake of ODN is now well documented. Depending on the ODN extracellular concentration, they may enter the cells either by specific absorptive endocytosis or by pinocytosis [29–31]. Although natural phosphodiester ODNs undergo a rapid extra-intracellular degradation [32,33], they can partially persist in the cell and rapidly reach the nucleus [12,34,35]. We demonstrated ODN cellular uptake in CEM-VLB100 by exposing the cells to 5'-labeled ODN. As illustrated in Fig. 2A, increasing amounts of cell-associated 1D was detected during the time of incubation. Similar results were obtained utilizing an ODN of the same length but with a different base composition (data not shown). Although with this labeling method the loss of labeled ^{32}P from the intact oligomer by cell-associated phosphatase activity can not be avoided, we calculated that approximately 0.1% of the total 1D supplied was associated with whole cells after 1 h of incubation at 37°C. Furthermore, we also demonstrated that by removing cell surface-bound ODN with acidic rinsing [35], undegraded 1D may be recovered from living cells after at least 4 h incubation at 37°C (Fig. 2B).

In order to investigate the effect of the triple helix on *mdr1* transcription we administered one dose of 1D ranging from 2.5 to 10 μM to CEM-VLB100 cells. Total RNA was extracted after 24 h incubation to avoid interference measurements due to a possible increase in MDR1 mRNA lifetime detected in MDR cell lines [36]. The Northern blot analyses, illustrated in Fig. 3A, demonstrate a significant, specific and dose-dependent reduction of MDR1 mRNA levels in 1D-treated cells with respect to untreated ones, by comparing the signals obtained from rehybridization of the same filters with the GPDH control probe. The same results were obtained utilizing a β-actin control probe (data not shown). Treatments with the inefficient triple helix-forming ODN 1E did not result in a variation in MDR1 mRNA levels with respect to untreated cells (Fig. 3B). Quantitation from seven independent experiments performed with 10 μM ODNs indicated a 46 ± 16% (S.D.) reduction in MDR1 mRNA in cells treated with 1D triple helix-forming ODN, with respect to untreated or 1E-treated controls.

Although sequence, orientation and type of administration of 1D seem incompatible with an antisense effect, we investigated the effect of cellular administration of a fully modified phosphorothioate 1D (1Ds). In fact, phosphorothioated ODNs are resistant to exo-endonuclease digestion and are thus suitable for antisense activity, retaining the ability to hybridize with mRNA [33,37]. However, phosphorothioate modification reduces the triplex stability and can completely impair triple helix formation [38,39]. In fact, the melting curve of 1Ds in the presence of its duplex W-C did not give the characteristic biphasic profile (Fig. 4A). As expected, considering the inability of 1Ds to form a triple helix, we did not detect a variation in MDR1 mRNA levels in 1Ds-treated CEM-VLB100 cells (Fig. 4B). For this reason we consider a potential antisense effect of 1D unlikely.

Taken together, these results seem to indicate that the natural unmodified triple helix-forming ODN 1D is able to inhibit, at least partially, MDR1 mRNA transcription in vivo. Since the DNA target is located in the coding region of the gene, we suggest that 1D may interfere with RNA polymerase activity.

4. Conclusions

ODN-directed intermolecular triple helix formation is one of the newest biotechnological approachies to allow site-specific targeting within megabase DNA. Small quantities of ODNs escaping intracellular degradation and persisting in to the nu-

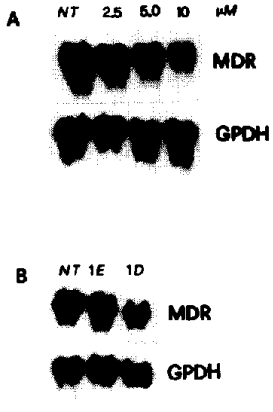

Fig. 3. MDR1 mRNA reduction by 1D. (A) Northern blot of total RNA extracted from 5×10^6 CEM-VLB100 cells, untreated (NT) or treated with 2.5–10 μM 1D as described in section 2. Autoradiography of the filter rehybridized with the GPDH control probe is also illustrated. (B) Northern blot of total RNA extracted from 5×10^6 CEM-VLB100 untreated (NT) or treated with 10 μM of 1D or 1E. The autoradiogram of the filter rehybridized with the GPDH control probe is also included.

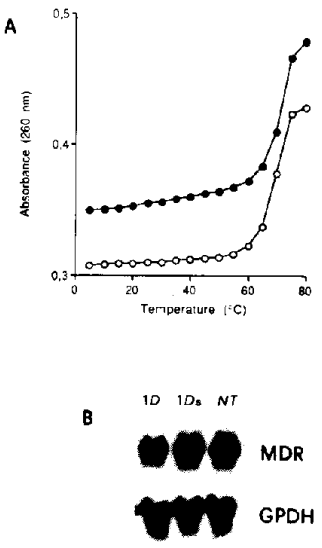

Fig. 4. Effect of fully phosphorothioated 1D (1Ds) on MDR1 mRNA levels. (A) Thermal denaturation curve. 4 nmol of the 35 bp synthetic duplex in 2 ml were annealed alone or with equimolar concentrations of 1Ds, as described in section 2. Absorbances at 260 nm of duplex alone and of duplex in the presence of 1Ds, are reported as a function of temperature (○, duplex; ●, duplex + 1Ds). (B) Northern blot of total RNA extracted from 5×10^6 CEM-VLB100 cells, untreated (NT) or treated with 10 μM of 1Ds or 1D, as described in section 2. Rehybridization of the filter with the control GPDH probe is illustrated.

cleus [12,34] may be sufficient to inhibit mRNA transcription. In fact, the target at the DNA level is lower than at the mRNA level, being the messenger continuously supplied by the transcription process.

Our results demonstrate the potential of a natural unmodified pyrimidine-rich triple helix-forming ODN to specifically reduce MDR1 mRNA levels in MDR cells. The specificity of 1D in recognizing its target is supported by the absence of an antisense effect and by the lack of effect on MDR1 mRNA levels in cells treated with an ODN with the same sequence but opposite orientation, unable to form a triple helix in vitro. Although we were unable to detect MDR1 truncated transcripts, since the probe recognizes a part of the gene downstream of the target exon 3 from 1178 to 2561 bp [25], we suspect that the triple helix can physically block the movement of RNA polymerase on the template DNA [8,41].

The partial reduction of MDR1 mRNA levels observed under our conditions might be related to the passive dissociation of 1D from the duplex, as seen in in vitro triple helix kinetics. In fact, melting curves we calculated that at 37°C only 20% of 1D was associated in a triplex form. Furthermore, we can not rule out the possibility that enzymatically susceptible unmodified ODN, reaching the nucleus intact may be insufficient for complexing all copies of the *mdr1* gene present in this drug-selected cell line [42].

The role of 1D in reducing MDR1 mRNA in MDR cell lines, associated to a low toxicity in animal models [20,21], could provide an interesting strategy for further investigation for the control of MDR cancer cells with respect to drug-resistant human tumor treatments.

Acknowledgements: This work was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC). The authors would like to thank Dr. Renata Lonigro for kindly providing β-actin and GPDH probes, Drs. Luigi Xodo, Mauro Giacca and Silvia Diviacco for many helpful discussions and suggestions, and Mr. Carlo Lo Cascio for the technical assistance to manuscript preparation.

References

[1] Felsenfeld, G., Davies, D.R. and Rich, A. (1957) J. Am. Chem. Soc. 79, 2023–2024.
[2] Hélène, C., Thuong, N.T., Saison-Behmoaras, T. and Francoise, J.C. (1989) Trends Biotechnol. 7, 310–315.
[3] Sun, J.-S. and Hélène, C. (1993) Curr. Opin. Struct. Biol. 3, 345–356.
[4] Chubbs, J.M. and Hogan, M.E. (1992) Trends Biotechnol. 10, 132–136.
[5] Maher, L.J. (1992) Bioassays 14, 807–815.
[6] Cooney, M., Czernuszewicz, G., Postel, E.H., Flint, S.J. and Hogan, M.E. (1988) Science 241, 456–459.
[7] Blume, S.W., Gee, J.E., Shrestha, K. and Miller, D.M. (1992) Nucleic Acids Res. 20, 1777–1784.
[8] Young, S.L., Krawczyk, S.H., Matteucci, M.D. and Toole, J.J. (1991) Proc. Natl. Acad. Sci. USA 88, 10023–10026.
[9] Duval-Valentin, G., Thuong, N.T. and Hélène, C. (1992) Proc. Natl. Acad. Sci. USA 89, 504–508.
[10] Wells, R.D., Collier, D.A., Hanvey, J.C., Shimizu, M. and Wohlrab, F. (1988) FASEB J. 2, 2939–2949.
[11] Behe, M.J. (1987) Biochemistry 26, 7870–7875.
[12] Postel, E.H., Flint, S.J., Kessler, D.J. and Hogan, M.E. (1991) Proc. Natl. Acad. Sci. USA 88, 8227–8231.
[13] Orson, F.M., Thomas, D.W., McShan, W.M., Kessler, D.J. and Hogan, M.E. (1991) Nucleic Acids Res. 19, 3435–3441.
[14] Rothernberg, M. and Ling, V. (1989) J. Natl. Cancer Inst. 81, 907–913.
[15] Fojo, A.T., Ueda, K., Slamon, D.J., Poplack, D.G., Gottesman, M.M. and Pastan, I. (1987) Proc. Natl. Acad. Sci. USA 84, 265–269.
[16] Michieli, M., Giacca, M., Fanin, R., Damiani, D., Geromin, A. and Baccarani, M. (1991) Br. J. Haematol. 78, 288–289.
[17] Lonn, U., Lonn, S., Nylen, U. and Stenkvist, B. (1992) Int. J. Cancer 51, 682–686.
[18] Abraham, E.H., Prat, A.G., Gerweck, L., Seneveratne, T., Arceci, R.J., Kramer, R., Guidotti, G. and Cantiello, H.F. (1993) Proc. Natl. Acad. Sci. USA 90, 312–316.
[19] Vlassov, V.V., Karamyshev, V.N. and Yakubov, L.A. (1991) FEBS Lett. 327, 271–274.
[20] Agrawal, S., Temsamani, J. and YangTang, J. (1991) Proc. Natl. Acad. Sci. USA 88, 7595–7599.
[21] Vlassov, V.V. and Yakubov, L.A. (1991) in: Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS (Wickstrom, E. ed.) pp. 243–266, Wiley-Liss, New York.
[22] Beck, W.T., Mueller, T.J. and Tanzer, L.R. (1979) Cancer Res. 39, 2070–2076.
[23] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[24] Chen, C., Chin, J.E., Ueda, K., Clark, D.P., Pastan, I., Gottesman, M.M. and Roninson, I.B. (1986) Cell 47, 381–389.
[25] Chen, C., Clark, D., Ueda, K., Pastan, I., Gottesman, M.M. and Roninson, I.B. (1990) J. Biol. Chem. 265, 506–514.
[26] Xodo, L.E., Manzini, G. and Quadrifoglio, F. (1990) Nucleic Acids Res. 18, 3557–3564.
[27] Xodo, L.E., Alunni-Fabbroni, M., Manzini, G. and Quadrifoglio, F. (1993) Eur. J. Biochem. 212, 395–401.
[28] Sun, J.-S., De Bizemont, T., Duval-Valentin, G., Montenay-Garestier, T. and Hélène, C. (1991) C.R. Acad. Sci. Paris Séries III 313, 585–590.
[29] Loke, S.L., Stein, C.A., Zhang, X.H., Mori, K., Nakanishi, M., Subasinghe, C., Cohen, J.S. and Neckers, L.M. (1989) Proc. Natl. Acad. Sci. USA 86, 3474–3478.
[30] Yakubov, L.A., Deeva, E.A., Zarytova, V.F., Ivanova, E.M., Ryte, A.S., Yurchenko, L.V. and Vlassov, V.V. (1989) Proc. Natl. Acad. Sci. USA 86, 6454–6458.

[31] Stein, C.A., Tonkinson, J.L., Zhang, L.-M., Yakubov, L., Gervasoni, J., Taub, R. and Rotenberg, S.A. (1993) Biochemistry 32, 4855–4861.
[32] Thierry, A.R. and Dritschilo, A. (1992) Nucleic Acids Res. 20, 5691–5698.
[33] Fisher, T.L., Terhorst, T., Cao, X. and Wagner, R.W. (1993) Nucleic Acids Res. 21, 3857–3865.
[34] Leonetti, J.P., Mechti, N., Degols, G., Gagnor, C. and Lebleu, B. (1991) Proc. Natl. Acad. Sci. USA 88, 2702–2706.
[35] Bergam, R., Connell, Y., Fahmy, B. and Neckers, L. (1993) Nucleic Acids Res. 21, 3567–3573.
[36] Gekeler, V., Frese, J., Diddens, H. and Probst, H. (1988) Biochem. Biophys. Res. Commun. 155, 754–760.
[37] Stein, C.A., Subasinghe, C., Shinozuka, K. and Cohen, J.S. (1988) Nucleic Acids Res. 16, 3209–3221.
[38] Kim, S.-G., Tsukahara, S., Yokoyama, S. and Takaku, H. (1992) FEBS Lett. 314, 29–32.
[39] Xodo, L.E., Alunni-Fabbroni, M., Manzini, G. and Quadrifoglio, F. (1994) Nucleic Acids Res. (in press).
[40] Yoshimura, A., Kuwazuru, Y., Sumizawa, J., Ikeda, S., Ichikawa, M., Usagawa, T. and Akiyama, S. (1989) Biochim. Biophys. Acta 992, 307–314.
[41] Sarkar, M.S. and Brahmachari, S.K. (1992) Nucleic Acids Res. 20, 5713–5718.
[42] Riordan, R., Deuchars, K., Kartner, N., Alon, N., Trent, J. and Ling, V. (1985) Nature 316, 817–819.