Communication

Peptide Transport by the Multidrug Resistance Pump*

(Received for publication, December 3, 1991)
Rakesh C. Sharma, Sachio Inoue, Joseph Roitelman, Robert T. Schimke, and Robert D. Simon‡
From the Department of Biological Sciences, Stanford University, Stanford, California 94305-5020

The membrane P-glycoprotein (P170) is an ATP-hydrolyzing transmembrane pump, and elevated levels of P170, due to higher expression with or without amplification of the multidrug resistance gene (mdrl), result in resistance to a variety of chemotherapeutic agents in mammalian cells. The function of the P170 pump has been proposed as a protection against toxic substances present in animal diets. Here we describe a Chinese hamster ovary cell line that was selected for resistance to a synthetic tripeptide, N-acetyl-leucyl-leucyl-norleucinal (ALLN). This ALLN-resistant variant shows the classical multidrug resistance (MDR) phenotype, including overexpression and amplification of the mdrl gene. Additionally, a mouse embryo cell line overexpressing the transfected mdrl gene is likewise resistant to ALLN. Our results demonstrate that P170 is capable of transporting peptides and raise the possibility that the mdrl gene product or other MDR-like genes, present in the genome of mammalian cells, may be involved in secretion of peptides or cellular proteins as is the case with the structurally similar hylB and ste6 gene products of Escherichia coli and yeast, respectively.

There exist in any number of species, from bacteria to man, a class of ATP-hydrolyzing transmembrane exit pumps that transport various intracellular compounds to the outside. These pumps bear varying degrees of structural or sequence homology. Many bacterial examples suggest a role in preventing accumulation of toxic agents (1, 2). A similar role is vented in mammalian cells, stably transfected with the chimeric gene HMGal (16), were treated with 5 µg/ml ALLN. These ALLN-resistant cells were replated as above with 50-100% increase in resistance, exponentially growing CHO-K1 cells were treated with 5 µg/ml ALLN. The medium containing ALLN was replaced every 2-3 days until cells grew to about 70% confluency, and at this stage cells were trypsinized, collected, and lysed in lysis buffer (1% deoxycholate, 5 mM EDTA, 5 mM EGTA in phosphate-buffered saline). Nuclei were removed by centrifugation (3,000 X g ~ 15 min), and proteins were resolved by 5-15% gradient SDS-polyacrylamide gel electrophoresis. Proteins were electroblotted onto nitrocellulose membrane, and the membranes were fixed with 0.2% Ponceau S in 5% trichloroacetic acid. The membranes were blocked with phosphate-buffered saline containing 5% nonfat milk for 1 h at room temperature. The membranes were then probed overnight with monoclonal anti-P170 antibody C219 (Centocor, Malvern, PA) or anti-calpain I1 antibody at 4 °C, washed, and incubated with horse-radish peroxidase-conjugated anti-mouse Ig antibody for 1 h at room temperature. The membranes were developed using an enhanced chemiluminescence Western blotting detection kit (Amersham Corp.) and exposed to x-ray film for 2-30 s. Genomic DNA was isolated from parental CHO-K1, a MDR cell line CHO CHC-5, and CHO-K1 ALLN™ cells by the standard method of phenol-chloroform extraction. MDR gene copy was estimated as described earlier (17).

EXPERIMENTAL PROCEDURES

Cell Culture, Drug Selection, and Cytotoxicity Assay—CHO-K1 cells, stably transfected with the chimeric gene HMGal (16), were grown in minimal essential medium containing dialyzed fetal bovine serum (10%), glutamine (2 mM), gentamicin (10 µg/ml), and G418 (250 µg/ml) at 37 °C in 5% CO2. For stepwise selection to ALLN resistance, exponentially growing CHO-K1 cells were treated with 5 µg/ml ALLN. This MDR phenotype is like genes, present in the genome of mammalian cells, may be involved in secretion of peptides or cellular proteins as is the case with the structurally similar hylB and ste6 gene products of Escherichia coli and yeast, respectively.

are involved in the outward transport of proteins including the hemolysin transport (HlyB) of Escherichia coli and transport of the Saccharomyces cerevisiae pheromone (α-factor), the ste6 protein (8, 9). Additionally the cystic fibrosis gene bears homology to the P170 protein (10).

The genomes of mammals contain, in addition to the mdrl gene, various numbers of mdrl-like genes of unknown function (11, 12). The question, thus, is raised as to whether the mdrl gene itself (generally associated with exclusion of toxic agents) or other members of this gene family in mammals may have a physiological function in outward transport of endogenous peptides (see Ref. 13 for a review of peptide or protein transport by such pumps). Here we provide evidence that the synthetic hydrophobic tripeptide, N-acetyl-leucyl-leucyl-norleucinal (ALLN) is a substrate for the P170 protein.

The reason for studying ALLN relates to its properties as an inhibitor of various intracellular proteases, including calpain I and II and cathepsins B, D, and E (14). Inoue et al. (15) reported that ALLN blocks the physiological degradation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in CHO cells. In an attempt to further understand the role of ALLN-sensitive protease(s) in the degradation of HMG-CoA reductase, we derived by stepwise selection CHO-K1 cells highly resistant to ALLN with the anticipation of amplifying gene(s) for the protease(s). The resistance phenotype obtained was related to overexpression and amplification of the mdrl gene.

* This work was supported in part by National Institutes of Health Grants HL 26502 (to R. D. S.) and CA 16518 (to R. T. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed.
‡ The abbreviations used are: MDR, multidrug resistance; ALLN, N-acetyl-leucyl-leucyl-norleucinal; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HMGal, fusion protein between the membrane domain of HMG-CoA reductase and E. coli β-galactosidase; CHO, Chinese hamster ovary; LDso, dose resulting in 50% lethality; EGTA, ethylendibis(oxyethylene)nitrilo)tetraacetic acid; SDS, sodium dodecyl sulfate.
RESULTS AND DISCUSSION

In order to investigate the MDR phenotype of ALLN<sup>50</sup> cells, we determined the cytotoxicity of ALLN and the pleiotropic drugs (colchicine, doxorubicin, etoposide) to ALLN<sup>50</sup> cells (Fig. 1). When compared with parental CHO-K1 cells, ALLN<sup>50</sup> cells were 50-, 50-, 20-, and 14-fold more resistant to ALLN, doxorubicin, colchicine, and etoposide, respectively (Fig. 1). These data show that ALLN<sup>50</sup> cells are highly resistant to the pleiotropic drugs involved in MDR. The ALLN<sup>50</sup> cell line did not show any cross-resistance to either trimerexate or methotrexate (data not shown) suggesting that the ALLN resistance phenotype does not overlap with antifolate resistance (18). We do not know why ALLN is toxic to cells. However, it is reasonable that its toxicity relates to its inhibition of endogenous protease(s).

The MDR phenotype results from overexpression of P170 which is encoded by the mdrl gene. To estimate cellular levels of P-glycoprotein, postnuclear extracts from parental CHO-K1 and ALLN<sup>50</sup> cells were analyzed by immunoblotting with specific antibodies (Fig. 2A). When the C219 monoclonal antibody to P-glycoprotein was used, a band with a Mr of 170,000 corresponding to P-glycoprotein is detected in extracts from ALLN<sup>50</sup> cells (lane 2). A longer exposure showed a band with the same molecular weight in the extract from wild type CHO-K1 cells (lane 3). The intensity of the band, as determined densitometrically, is more than 10-fold higher in the ALLN<sup>50</sup> cell extract than in wild type CHO-K1 cell extract. When monoclonal anti-rabbit calpain II antibody was used, a band with a Mr of 80,000 is detected (lanes 5 and 6). The intensity of this band was the same in both cell extracts, indicating that the amount of calpain II did not change in ALLN-resistant cells. When antibodies against HMG-CoA reductase or annexin II were used to probe immunoblots, the same amounts of these proteins were detected in both cell extracts (data not shown). These results demonstrate that P170 is specifically overexpressed in ALLN<sup>50</sup> cells.

The increase in the level of P170 is not always accompanied by MDR gene amplification. However, most of the highly resistant stable MDR cell lines do show MDR gene amplification accompanied by a higher level of P170 (19). To determine whether the mdrl gene is amplified in a ALLN-resistant cell line, we isolated genomic DNA from parental CHO-K1, ALLN<sup>50</sup>, and a well characterized MDR cell line CHO CHC-5 (20, 21), subjected genomic DNA to digestion with EcoRI, and analyzed it by Southern blot (Fig. 2B) Amplification of the mdrl gene was detected in ALLN-resistant as well as in CHO CHC-5 cell lines (note: 5-fold less DNA was loaded for this latter cell line). As a control, the nylon membrane was stripped of washing in 0.1 × SSPE, 0.1% SDS at 85–95 °C and repored with c-myc DNA (22). Wild-type CHO-K1 and ALLN<sup>50</sup> cells showed similar hybridization to c-myc (data not shown). The amount of mdrl DNA present in these cells was quantitated by the slot-blot technique (23) using c-myc DNA as a control gene. Densitometric scanning of exposed films show about 10-fold amplification of mdrl gene in ALLN<sup>50</sup> cells (data not shown). Thus, the elevated levels of P170 could be accounted for solely by mdrl gene amplification.

The typical MDR phenotype which includes: (i) cross-resistance to pleiotropic drugs like doxorubicin, colchicine, etoposide; (ii) increased expression of membrane-associated P-glycoprotein; (iii) higher expression of P-glycoprotein with mdrl gene amplification, is found in the ALLN<sup>50</sup> cells. Characteristically, the MDR phenotype is reversed by various agents, including verapamil (24) and quinidine (25). Verapamil binds to P-glycoprotein and reverses the MDR phenotype by a mechanism that involves competitive inhibition of drug transport by P-glycoprotein (26–28). Fig. 3 shows that verapamil (10 μM) reverses the ALLN resistance phenotype of ALLN<sup>50</sup> cells by increasing their sensitivity (35-fold) to ALLN. Verapamil results in a 2-fold increase in sensitivity to ALLN in parental CHO-K1 cells. This is consistent with results obtained with other wild-type cell lines and pleiotropic drugs (25). Verapamil alone (up to 30 μM) has no effect on the viability of wild-type or ALLN<sup>50</sup> cells (data not shown). These observations are consistent with the conclusion that ALLN is a substrate for P-glycoprotein.

The above results suggest that the apparent resistance to ALLN is, in fact, a manifestation of lower steady-state levels of ALLN in ALLN<sup>50</sup> cells due to the outward pumping activity of P-glycoprotein. If this is the case, one would predict lower intracellular steady-state levels of ALLN in ALLN<sup>50</sup> cells to affect the mevalonate-accelerated degradation of HMGal. To test this possibility, we determined the effect of ALLN on the mevalonate-accelerated degradation of HMGal. Fig. 4 shows that the activity of HMGal in both cell lines drops to ~15% of control when the cells are given excess mevalonate. This decrease in activity is due to accelerated degradation of HMGal (15, 29). Addition of ALLN to the parental CHO-K1 cells causes a dose-dependent increase in HMGal activity to more than 90% of control levels, indicating that degradation was effectively blocked by ALLN with an IC<sub>50</sub> of 6 μg/ml (see also Ref. 15). In the ALLN<sup>50</sup> cells, however, ALLN inhibited the mevalonate-accelerated degradation of HMGal with an
copy number. For immunoblot analysis, 100 µg of protein of postnuclear extracts from CHO-K1 (lanes 1, 3, and 5) and ALLN<sup>500</sup> cells (lanes 2, 4, and 6) were resolved by SDS-polyacrylamide gel electrophoresis and were electroblotted onto nitrocellulose membrane. The membranes were then probed overnight with monoclonal anti-P170 antibody C219 (Centocor, Malvern, PA) membrane. The membranes were then probed overnight with monoclonal anti-calpain antibody (lanes 1-4) or anti-calpain II antibody (lanes 5 and 6) at 4 °C, washed, and incubated with horseradish peroxidase-conjugated anti-mouse Ig antibody for 1 h at room temperature. The membranes were washed, and blots were developed using an enhanced chemiluminescence Western blotting detection kit (Amersham Corp.) and exposed to x-ray film for 2-30 s. Lanes 3 and 4 are longer exposures of lanes 1 and 2. Molecular mass markers (in kDa) are indicated on the left. B, to estimate MDR genomic DNA by Southern blot analysis, high molecular weight genomic DNA (10 µg/lane, except for lane C, 5-µg sample) was digested to completion with EcoRI, fractionated on an 0.8% agarose gel, and blotted onto a Zetabind membrane. Blots were hybridized with a <sup>32</sup>P-oligolabeled CHO MDR probe (0.66-kilobase pair EcoRI fragment of the MDR cDNA clone p-CHP-1). Hybridization was carried out at 42 °C for 48 h in 50% formamide, 5 × saline/sodium phosphate/EDTA (SSPE), 1% SDS, and 1 × Denhardt's solution. The filter was given two 30-min high stringency washes in 0.1 × SSPE, 0.1% SDS at 65 °C and exposed for autoradiography (lanes 4-6). Ethidium bromide staining of the fractionated DNA is shown to confirm the amount of DNA loaded on the gel (lanes 1-3).

A

FIG. 2. Quantification of P-glycoprotein and MDR gene copy number. A, for immunoblot analysis, 100 µg of protein of postnuclear extracts from CHO-K1 (lanes 1, 3, and 5) and ALLN<sup>500</sup> cells (lanes 2, 4, and 6) were resolved by SDS-polyacrylamide gel electrophoresis and were electroblotted onto nitrocellulose membrane. The membranes were then probed overnight with monoclonal anti-P170 antibody C219 (Centocor, Malvern, PA) membrane. The membranes were then probed overnight with monoclonal anti-calpain antibody (lanes 1-4) or anti-calpain II antibody (lanes 5 and 6) at 4 °C, washed, and incubated with horseradish peroxidase-conjugated anti-mouse Ig antibody for 1 h at room temperature. The membranes were washed, and blots were developed using an enhanced chemiluminescence Western blotting detection kit (Amersham Corp.) and exposed to x-ray film for 2-30 s. Lanes 3 and 4 are longer exposures of lanes 1 and 2. Molecular mass markers (in kDa) are indicated on the left. B, to estimate MDR genomic DNA by Southern blot analysis, high molecular weight genomic DNA (10 µg/lane, except for lane C, 5-µg sample) was digested to completion with EcoRI, fractionated on an 0.8% agarose gel, and blotted onto a Zetabind membrane. Blots were hybridized with a <sup>32</sup>P-oligolabeled CHO MDR probe (0.66-kilobase pair EcoRI fragment of the MDR cDNA clone p-CHP-1). Hybridization was carried out at 42 °C for 48 h in 50% formamide, 5 × saline/sodium phosphate/EDTA (SSPE), 1% SDS, and 1 × Denhardt's solution. The filter was given two 30-min high stringency washes in 0.1 × SSPE, 0.1% SDS at 65 °C and exposed for autoradiography (lanes 4-6). Ethidium bromide staining of the fractionated DNA is shown to confirm the amount of DNA loaded on the gel (lanes 1-3).

B

FIG. 3. Effect of verapamil on the cytotoxicity of ALLN in parental CHO-K1 and ALLN-resistant cells. Exponentially growing CHO-K1 (○, ■) and ALLN<sup>500</sup> (□, ▲) cells were trypsinized and plated in medium containing various concentrations of ALLN alone or combined with verapamil (10 µM). Colonies were fixed and counted as described for Fig. 1. ○, □, ALLN alone; ○, ▲, ALLN plus verapamil.

FIG. 4. Lower steady-state levels of ALLN in ALLN<sup>90</sup> cells. CHO-K1 and ALLN<sup>500</sup> cells, both transfected with HMGal, were grown in 24-well dishes in minimal essential medium containing 5% lipid-poor serum and 250 µg/ml G418. On Day 1, cells were refed with the same medium supplemented also with 1 µM compactin and 100 µM mevalonate (29). On Day 2, ALLN was added in dimethyl sulfoxide (final concentration, 1% v/v) to the indicated concentrations. Mevalonate (20 mM) was added 1 h after the addition of ALLN, and residual HMGal activity was determined 22 h later, as described under "Experimental Procedures." The results, expressed as percent of HMGal activity remaining in the presence of mevalonate relative to control cells receiving no mevalonate, are the mean of two separate experiments each performed in hexaplicate incubations for every ALLN concentration. ALLN is not soluble in aqueous solution at concentrations higher than 200 µg/ml.

FIG. 5. Cytotoxicity of ALLN to parental NIH 3T3 and 3T3 pHaMDR1 cells. The parental NIH 3T3 (○) cells and NIH 3T3 cells transfected with a wild-type MDR1 cDNA (3T3 pHaMDR1/6A Cl 2-1) (■) were obtained from Michael M. Gottesman, NIH. Cells were grown in minimal essential medium supplemented with 10% fetal calf serum, 10 µg/ml gentamicin, and 2 mM glutamine. Exponentially growing cells were trypsinized and plated (1 × 10<sup>3</sup> cells/6-cm dish) in 5 ml of complete media containing various amounts of ALLN. After 6-8 days of incubation, colonies were fixed and counted as described in Fig. 1.

IC<sub>50</sub> of 90 µg/ml, and almost full inhibition was achieved at approximately 200 µg/ml (Fig. 4). At concentrations higher than 200 µg/ml ALLN starts precipitating. Since in the absence of ALLN, HMGal is degraded to a similar extent in both cell lines, these results indicate that the machinery responsible for the regulated degradation of HMGal was unaffected during selection for ALLN resistance. Therefore, the higher IC<sub>50</sub> for inhibition of HMGal degradation reflects lower steady-state concentrations of ALLN in ALLN<sup>90</sup> cells. This
is consistent with the conclusion that ALLN is pumped out of the cells by P-glycoprotein.

The data described above show that stepwise selection with ALLN gives rise to a typical MDR cell line. Therefore, one would predict that a MDR cell line that has been developed by selecting with other MDR drugs (e.g. colchicine) should also be resistant to ALLN. To test this, we have identified MDR cell line, CHO CHF-5, that was stepwise selected for resistance to colchicine (20, 21) was employed. The cytotoxicity data show that CHF-5 cells are as resistant to ALLN as are ALLN cells (LD50 = 94 and 92 µg/ml, respectively, data not shown).

Within the chromosomal region amplified in many CHO cells subjected to selection for MDR phenotype is a series of mdr-like genes, i.e. they have sequence homology to the well studied mdr1 gene (11, 12). The function of these genes is little understood, and the question arises as to whether the resistance to ALLN is specifically due to overexpression of the mdr1 gene or whether it may actually result from co-amplification of an mdr-like gene, i.e. the selection is not actually on the mdr1 gene per se. To rule out this possibility we have employed a mouse cell line, NIH 3T3 pHaMDR1/6A, transfected with wild-type mdr1 cDNA. The plasmid pHaMDR1/6A confers the MDR phenotype on these mouse cells (30, 31). Fig. 5 shows that NIH 3T3 cells transfected with the mdr1 gene (3T3 pHaMDR1) are highly resistant to ALLN with a 10-fold higher LD50 than the parental NIH 3T3 cells. Since 3T3 pHaMDR1 cells show the MDR phenotype, we conclude that the tripeptide ALLN is, indeed, a substrate for P-glycoprotein. Although other MDR cell line cells with or without mdr1 gene amplification have been reported to be cross-resistant to cyclic peptides (32–34), to the best of our knowledge this is the first report demonstrating MDR phenotype and gene amplification resulting from selection with a linear peptide.

The present study shows that the MDR pump is capable of transporting peptide(s). Considering the homology of P170 with hemolysin transport protein (Hy1B) of E. coli, the Sec6 protein of yeast, and the putative endoplasmic reticulum transporter proteins involved in antigen presentation (35, 36), it is reasonable to suggest that one of the physiological functions of P-glycoprotein and its related genes may be the secretion of peptides and cellular proteins from mammalian cells.

Acknowledgments—We thank Michael M. Gottesman for providing NIH 3T3 and 3T3 pHaMDR1 cell lines, V. Ling for the CHO CHF-5 cell line, S. Kawashima for calpain antibody, and S. Bar-Nun for helpful discussions and critical review of the manuscript.

REFERENCES
1. Ames, G. F.-L., Mimura C. S., and Shyamala, V. (1990) FEMS Microbiol. Rev. 6, 429-446
2. Ames, G. F.-L. (1996) Annu. Rev. Biochem. 55, 397-425
3. Fojo, A. T., Ueda, K., Slamon, D. J., Poplack, D. G., Gottesman, M. M., and Pastan, I. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 265-269
4. Arceci, R. J., Croop, J. M., Horwitz, S. B., and Housman, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4550-4554
5. Sugawara, I., Kataoka, I., Morishita, Y., Hamada, H., and Tsuruo, T. (1988) Cancer Res. 48, 1926-1929
6. Gottesman, M. M., and Pastan, I. (1988) J. Biol. Chem. 263, 12163-12166
7. Endicott, J. A., and Ling, V. (1989) Annu. Rev. Biochem. 58, 137-171
8. McGrath, J. F., and Varshavsky, A. (1989) Nature 340, 400-403
9. Kucher, K., Sterne, R. E., and Thormer, J. (1989) EMBO J. 8, 3975-3984
10. Riorian, J. R., Rommens, J. M., Keren, B. S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J. L., Drumm, M. L., Jannuzzi, M. C., Collins, F. S., and Tsai, L. C. (1989) Science 245, 1066-1072
11. Van der Bliek, A. M., Van der Veldekoerts, T., Ling, V., and Borst, P. (1986) Mol. Cell. Biol. 6, 1671-1678
12. Brujin, M. H. L., Van der Bliek, A. M., Biedler, J. L., and Borst, P. (1986) Mol. Cell. Biol. 6, 4717-4722
13. Kucher, K., and Thormer, J. (1990) Curr. Opin. Cell Biol. 2, 617-624
14. Hisawa, T., Sawada, T., and Sakiyama, Y. (1990) Carcinogenesis 11, 75-80
15. Inoue, S., Bar-Nun, S., Boitelman, J., and Simoni, R. D. (1991) J. Biol. Chem. 266, 13311-13317
16. Skalnik, D. G., Narita, H., Kent, C., and Simoni, R. D. (1989) J. Biol. Chem. 264, 6836-6841
17. Sharma, R. C., Assaraf, Y. G., and Schimke, R. T. (1991) Cancer Res. 51, 2949-2959
18. Assaraf, Y. G., Molina, A., and Schimke, R. T. (1989) J. Biol. Chem. 264, 18326-18334
19. Shen, D. W., Fojo, A., Chin, J. E., Roninson, I. B., Richert, N., Pastan, I., and Gottesman, M. M. (1988) Science 232, 643-645
20. Ling, V., and Thompson, L. H. (1974) J. Cell. Biol. 63, 105-116
21. Bech-Hansen, N. T., Till, J. E., and Victor, L. (1976) J. Cell. Physiol. 88, 23-32
22. Shen-Ong, G. L. C., Keath, E. J., Piccolo, S. P., and Cole, M. D. (1982) Cell 31, 443-452
23. Brown, P. C., Tlsty, T. D., and Schimke, R. T. (1989) Mol. Cell. Biol. 3, 1097-1107
24. Tsuruo, T., Lida, H., Tsukagoshi, S., and Sakurai, Y. (1981) Cancer Res. 41, 1967-1972
25. Fojo, A., Akiyama, S., Gottesman, M. M., and Pastan, I. (1985) Cancer Res. 45, 3002-3007
26. Cornwell, M. M., Safa, A., Felted, R. L., Gottesman, M. M., and Pastan, I. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3847-3850
27. Safa, A. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7187-7191
28. Yusa, K., and Tsuruo, T. (1989) Cancer Res. 49, 5002-5006
29. Chun, K. T., Bar-Nun, S., and Simoni, R. D. (1991) J. Biol. Chem. 266, 22004-22010
30. Pastan, I., Gottesman, M. M., Ueda, K., Lovelace, E., Rutherford, A. V., and Willingham, M. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4486-4490
31. Ueda, K., Cardarelli, C., Gottesman, M. M., and Pastan, I. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5004-5008
32. Cole, P. C. S., Chanda, E. R., Dicke, F. P., Gerlach, J. H., and Mirkis, E. L. (1991) Cancer Res. 51, 3345-3352
33. Baas, F., Jongsma, A. P. M., Broxterman, H. J., Arceci, R. J., Housman, D., Scheffer, G. L., Riethorst, A., Groenen, M. V., Niewint, A. W. M., and Joenje, H. (1990) Cancer Res. 50, 5392-5398
34. Daoud, S. S., and Juliano, R. L. (1989) Cancer Res. 49, 2661-2667
35. Peterson, E. V., Gow, J. R., Coadwell, W. J., Monaco, J. J., Butcher, G. W., and Howard, J. C. (1990) Nature 348, 738-741
36. Trowdale, J. Hanson, I., Mockridge, I., Beck, S., Townsend, A., and Kelly, A. (1990) Nature 348, 741-744