**An Antisense Oligodeoxynucleotide That Depletes RI_α Subunit of Cyclic
AMP-dependent Protein Kinase Induces Growth Inhibition in Human** _{ICANCER RESEARCH 53.868–872. February 15. 1993]
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Cellular Biochemistry Section (H. Y. A.**

**Hiroshi Yokozaki, Alfredo Budillon, Giampaolo Tortora, Scott Meissner, Serge L. Beaucage, Keizaburo Miki, and
Yoon S. Cho-Chung¹
Cellular Biochemistry Section [H. Y., A. B., G. T., Y. S. C-C.] and Experimental Oncology Hiroshi Yokozaki, Alfredo Budillon, Giampaolo Tortora, Scott Meissner, Serge L. Beaucage, I
Yoon S. Cho-Chung¹
Cellular Biochemistry Section [H. Y., A. B., G. T., Y. S. C-C.] and Experimental Oncology Section [S. M.], L**

ABSTRACT

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** Enhanced expression of the RI_{α} subunit of cyclic AMP-dependent pro-
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evidence that $\mathbb{R}I_{\alpha}$ is a growth-inducing protein that may be essential for
neoplastic cell growth. Human colon, breast, and gastric carcino evidence that RI_a is a growth-inducing protein that may be essential for
neoplastic cell growth. Human colon, breast, and gastric carcinoma and
neuroblastoma cell lines exposed to a 21-mer human RI_a antisense phos-
with
 neoplastic cell growth. Human colon, breast, and gastric carcinoma and neuroblastoma cell lines exposed to a 21-mer human RI_{ α **} antisense phos-
phorothioate oligodeoxynucleotide (S-oligodeoxynucleotide) exhibited grow neuroblastoma cell lines exposed to a 21-mer human RI**_{α} antisense phos-
phorothioate oligodeoxynucleotide (S-oligodeoxynucleotide) exhibited
growth inhibition with no sign of cytotoxicity. Mismatched sequence (ran-
 phorothioate oligodeoxynucleotide (S-oligodeoxynucleotide) exhibited of growth inhibition with no sign of cytotoxicity. Mismatched sequence (random) S-oligodeoxynucleotides of the same length exhibited no effect. The the growth inhibition with no sign of cytotoxicity. Mismatched sequence (rancell dom) S-oligodeoxynucleotides of the same length exhibited no effect. The the expowth inhibitory effect of RI_{α} antisense oligomer correlated **inhibition S-oligodeoxynucleotides of the same length exhibited no effect. The** the growth inhibitory effect of RI_{α} antisense oligomer correlated with a decrease in the RI_{α} mRNA and protein levels and with an inc **growth inhibitory effect of RI_{** α **} antisense oligomer correlated with a de-** site-selectic crease in the $\mathbf{R}\mathbf{I}_{\alpha}$ mRNA and protein levels and with an increase in RII_B cell lines (the regulatory subunit of pro **antisense in the RI_a mRNA and protein levels and with an increase in RII_B cell lines (the regulatory subunit of protein kinase type II) expression. The growth kinase typerinhibition was abolished, however, when cells** (the regulatory subunit of protein kinase type II) expression. The growth inhibition was abolished, however, when cells were exposed simultaneously to both RI_{α} and RII_{β} antisense S-oligodeoxynucleotides. The $RII_{\$ inhibition was abolished, however, when cells were exposed simultaneously to both RI_{α} and $RIII_{\beta}$ antisense S-oligodeoxynucleotides. The RII_{β} was antisense S-oligodeoxynucleotide alone, exhibiting suppression of neously to both RI_{α} and RII_{β} antisense S-oligodeoxynucleotides. The RII_{β} antisense S-oligodeoxynucleotide alone, exhibiting suppression of RII_{β} along with enhancement of RI_{α} expression, led to slight s antisense S-oligodeoxynucleotide alone, exhibiting suppression of RII_B and along with enhancement of RI_α expression, led to slight stimulation of cell and growth. These results demonstrate that two isoforms of cyclic along with enhancement of RI_{α} expression, led to slight stimulation of cell growth. These results demonstrate that two isoforms of cyclic AMP receptor proteins, RI_{α} and $RIII_{\beta}$, are reciprocally related in the gr growth. These results demonstrate that two isoforms of cyclic AMP receptor proteins, $\mathbf{R}\mathbf{I}_{\alpha}$ and $\mathbf{R}\mathbf{II}_{\beta}$, are reciprocally related in the growth control of cancer cells and that the $\mathbf{R}\mathbf{I}_{\alpha}$ anti iciently depletes the growth stimulatory \mathbf{RI}_{α} , is a powerful biological cells
bitoward suppression of malignancy.
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cAMP² functions as a transducer of hormonal signals through bind-
g to its recepto

INTRODUCTION

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cAMP² functions as a transducer of hormonal signals through bind-
ing to its receptor protein, cAMP-dependent protein kinase (1). The
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growth in the same different component protein kinase (1). The
tetrameric protein kinase is composed of two C subunits bound to a R
subunit dimer. Activation occurs when two cAMP molecules bind to
a The 2 **INTRODUCTION** grow

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tetrameric protein kinase is composed of two C subunits bound cAMP² functions as a transducer of hormonal signals through bind-
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subunit dimer. Activation occurs when two cAMP molecules bind to
each regulatory subunit of protein kinase, resulting in the release of a
c subunit. Th subunit dimer. Activation occurs when two cAMP molecules bind to
each regulatory subunit of protein kinase, resulting in the release of a
c subunit. Thus, all actions of cAMP as second messenger that are 8700
implicated i each regulatory subunit of protein kinase, resulting in the release of a
C subunit. Thus, all actions of cAMP as second messenger that are 8700
implicated in the regulation of various cellular functions, such as performet C subunit. Thus, all actions of cAMP as second messenger that are applicated in the regulation of various cellular functions, such as permetabolism, secretion, cell proliferation, ion channel regulation, and quene inducti implicated in the regulation of various cellular functions, such as
metabolism, secretion, cell proliferation, ion channel regulation, and
gene induction, have been related to protein phosphorylation through
the activatio imetabolism, secretion, cell pro
gene induction, have been relate
the activation of protein kinase
the presence of an R subunit in
inhibition of the C subunit.
There are, however, two type Example, the production, the calculation and
the induction, have been related to protein phosphorylation through
activation of protein kinase (2). Consequently, the significance of
initiation of the C subunit.
There are, the activation of protein kinase (2). Consequently, the significance of mism
the presence of an R subunit in protein kinase has been confined to its
inhibition of the C subunit.
There are, however, two types of cAMP-depen

the presence of an R subunit in protein kinase has been confined to its
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There are, however, two types of cAMP-dependent protein kinases,
type I and type II, in mammalian cells. These kinases a The suburit (3). There are, however, two types of cAMP-dependent protein kinases, type I and type II, in mammalian cells. These kinases are distinguished My their different R subunits, RI and RII, that interact with an id There are, however, two types of cAMP-dependent protein kinases,

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by their different R subunits, RI and RII, that interact with an identical
C subunit (3). Through biochemical studies and gene cloning, four
isofo by their different R subunits, RI and RII, that interact with an identical cals,
C subunit (3). Through biochemical studies and gene cloning, four macis
isoforms of the R subunits, RI_{α} , RI_{β} , $RI_{I_{\alpha}}$, and RI_{I_{β C subunit (3). Through biochemical studies and gene cloning, four macial sisoforms of the R subunits, RI_{α} , RI_{β} , $RI_{I_{\alpha}}$, and $RI_{I_{\beta}}$, have been inactified (4, 5). These R isoforms differ in tissue distribution throughout the cytoplasm, whereas RII localizes to nuclei, nucleoli,
and in biochemical properties (3, 8). The two general isoforms of the
R subunit also differ in their subcellular localization. RI is found
throughout th subunit also differ in their subcellular localization. RI is found

coughout the cytoplasm, whereas RII localizes to nuclei, nucleoli,
 $\frac{1}{3}$.

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Institute; and Food and Drug Administration [S. L. B.], Bethesda, Maryland 20892; Terumo Corporation [K. M.], Kanagawa, Japan
 ABSTRACT
 Enhanced expression of the RI_a subunit of cyclic AMP-dependent pro
 Enhanced e gy Section [S. M.], Laboratory of Tumor Immunology and Biology, National Cancer
Corporation [K. M.], Kanagawa, Japan
Golgi, and to the microtubule-organizing center (9–12). Thus, these
studies suggest specific roles of the gy Section [S. M.], Laboratory of Tumor Immunology and Biology, National Cancer
Corporation [K. M.], Kanagawa, Japan
Golgi, and to the microtubule-organizing center (9–12). Thus, these
studies suggest specific roles of the Corporation $(K, M, J, Kanagawa, Japan)$
Golgi, and to the microtubule-organizing center (9–12). Thus, these
studies suggest specific roles of the different isoforms of the R sub-
unit, apart from inhibition of the C subunit in the reg Golgi, and to the microtubule-organizing center $(9-12)$. Thus, these studies suggest specific roles of the different isoforms of the R subunit, apart from inhibition of the C subunit in the regulatory function of cAMP. A Golgi, and to the microtubule-organizing center (9–12). Thus, these studies suggest specific roles of the different isoforms of the R sub-
unit, apart from inhibition of the C subunit in the regulatory function
of cAMP. A studies suggest specific roles of the different isoforms of the R sub-
studies suggest specific roles of the different isoforms of the R sub-
unit, apart from inhibition of the C subunit in the regulatory function
of cAMP unit, apart from inhibition of the C subunit in the regulatory function
of cAMP. An enhanced expression of RI_{α} subunit has been shown (12,
13) in human cancer cell lines and in primary tumors, as compared
with normal of cAMP. An enhanced expression of RL_a subunit his been shown (12, 13) in human cancer cell lines and in primary tumors, as compared with normal counterparts, in cells after transformation with the Ki-ras oncogene or tr 13) in human cancer cell lines and in primary tumors, as compared
with normal counterparts, in cells after transformation with the Ki-ras
oncogene or transforming growth factor α , and upon stimulation of
cell growth wi For in numan cancer centries and in primary dimots, as compared
with normal counterparts, in cells after transformation with the Ki-ras
oncogene or transforming growth factor α , and upon stimulation of
cell growth with oncogene or transforming growth factor α , and upon stimulation of cell growth with GM-CSF or phorbol esters; conversely, a decrease in the expression of RI_{α} correlates with growth inhibition induced by site-sele cell growth with GM-CSF or phorbol esters; conversely, a decrease in the expression of RI_{α} correlates with growth inhibition induced by site-selective cAMP analogues in a broad spectrum of human cancer cell lines (13) the expression of $\mathbf{R}I_{\alpha}$ correlates with growth inhibition incisite-selective cAMP analogues in a broad spectrum of huma
cell lines (13). Moreover, an inverse relationship between RI
kinase type I) and RII (protein Expression of Eq. correlates whill growth inhibition induced by
e-selective cAMP analogues in a broad spectrum of human cancer
Il lines (13). Moreover, an inverse relationship between RI (protein
nase type I) and RII (pro

cell lines (13). Moreover, an inverse relationship between RI (protein kinase type I) and RII (protein kinase type II) has been noted during ontogenic development and cell differentiation (12, 13). We hypothesize that $RI_{$ kinase type I) and RII (protein kinase type II) has been noted during
ontogenic development and cell differentiation (12, 13).
We hypothesize that RI_{α} is an ontogenic growth-inducing protein,
and its constitutive exp ontogenic development and cell differentiation (12, 13).
We hypothesize that RI_{α} is an ontogenic growth-inducing protein,
and its constitutive expression disrupts normal ontogenic processes,
resulting in a pathogenic We hypothesize that $\mathbf{R}\mathbf{I}_{\alpha}$ is an ontogenic growth-inducing protein,
and its constitutive expression disrupts normal ontogenic processes,
resulting in a pathogenic outgrowth, such as malignancy. To directly
deter and its constitutive expression disrupts normal ontogenic processes, resulting in a pathogenic outgrowth, such as malignancy. To directly determine whether RI_{α} has a role in neoplastic cell growth, at least *in v* resulting in a pathogenic outgrowth, such as malignancy. To directly
determine whether RI_{α} has a role in neoplastic cell growth, at least in
vitro, we used the antisense strategy (14). We exposed human cancer
cells to determine whether RI_{α} has a role in neoplastic cell growth, at least *in vitro*, we used the antisense strategy (14). We exposed human cancer cells to an antisense unmodified oligodeoxynucleotide (15) and to a ph *vitro*, we used the antisense strategy (14). We exposed human cancer cells to an antisense unmodified oligodeoxynucleotide (15) and to a phosphorothioate analogue complementary to the first 21 bases of the human RI_{α} cells to an antisense unmodified oligodeoxynucleotide (15) and to implosphorothioate analogue complementary to the first 21 bases of the human RI_{α} mRNA and examined the effects of this treatment on cel growth and morp human RI_{α} mRNA and examined the effects
growth and morphology. As controls, we us
(random) and RI_{β} antisense (16) oligomers
MATERIALS AND METHODS

mdom) and RII_B antisense (16) oligomers of the same length.
 ATERIALS AND METHODS

The 21-mer oligodeoxynucleotides and their phosphorothioate analogues

ad in the present study were synthesized (17) using a Milligen MATERIALS AND METHODS
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used in the present study were synthesized (17) using a Milligen Biosearch
8700 DNA synthesizer (Bedford, MA) and purified by reve MATERIALS AND METHODS
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8700 DNA synthesizer (Bedford, MA) and purified by reverse-phase high-
perfor 8700 DNA synthesizer (Bedford, MA) and purified by reverse-phase high-
performance liquid chromatography. The oligomers had the following se-
quences: human RI_{α} antisense, 5'-GGC-GGT-ACT-GCC-AGA-CTC-CAT-3'
(15); $RIII_{\$ performance liquid chromatograpuences: human RI_α antisense, (15); RII_β antisense, 5'-CGC-CG mismatched sequence, random onucleotides at every position.
R-Cl-cAMP was obtained from ences: human RI_a antisense, 5'-GGC-GGT-ACT-GCC-AGA-CTC-CAT-3'

(i); RII_B antisense, 5'-CGC-CGG-GAT-CTC-GAT-GCT-CAT-3' (16); and the

smatched sequence, random oligomer was a random mixture of all four

cleotides at eve

(15); RII_B antisense, 5'-CGC-CGG-GAT-CTC-GAT-GCT-CAT-3' (16); and the mismatched sequence, random oligomer was a random mixture of all four nucleotides at every position.
8-CI-CAMP was obtained from the Division of Canc mismatched sequence, random oligomer was a random mixture of all four
nucleotides at every position.
8-Cl-cAMP was obtained from the Division of Cancer Treatment, NCI
(Bethesda, MD). Pepstatin, antipain, chymostatin, leupe 8-C1-cAMP was obtained from the Division of Cancer Treatment, NCI
(Bethesda, MD). Pepstatin, antipain, chymostatin, leupeptin, and soybean
trypsin inhibitor were obtained from Sigma Chemical Company (St. Louis,
MO). 8-N₃ trypsin inhibitor were obtained from Sigma Chemical Company (St. Louis, MO). 8-N₃-[³²P]cAMP (60.0 Ci/mmol) was obtained from ICN Pharmaceuticals, Inc. (Irvine, CA). Protein A-Sepharose CL-4B was purchased from Pharmaci MO). 8-N₃-[³²P]cAMP (60.0 Ci/mmol) was obtained from ICN Pharmaceuticals, Inc. (Irvine, CA). Protein A-Sepharose CL-4B was purchased from Pharmacia-LKB (Uppsala, Sweden). Eagle's minimum essential medium, heatinactivat cals, Inc. (Irvine, CA). Protein A-Sepharose CL-4B was purchased from Pharmacia-LKB (Uppsala, Sweden). Eagle's minimum essential medium, heatinactivated fetal bovine serum, trypsin-EDTA solution, penicillin-streptomycins o macia-LKB (Uppsala, Swede

inactivated fetal bovine serum

solution, L-glutamine, HEPES

mum essential medium nones

BRL (Grand Island, NY).

Cell Culture. The LS-174 **CEAL CULTURE CONDEG CONDEG CONDEG CONDEG CONDENSIGED** CONDECITE UNIVERSITY (UNITELNATION, L-glutamine, HEPES buffer (1 M stock solution, pH 7.3), and minimum essential medium nonessential amino acids were obtained from GI

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18 U.S.C. Section 1734 solely to indicate this f Solution, L-glutamine, HEPES buffer (1 M stock solution, pH 7.3), and mini-
mum essential medium nonessential amino acids were obtained from GIBCO-
BRL (Grand Island, NY).
Cell Culture. The LS-174T human colon carcinoma ce minimum essential medium colon carcinoma cell line (provided by

1. Greiner, National Cancer Institute) and SK-N-SH human neuroblastoma cell

line (provided by L. Neckers, National Cancer Institute) were grown in Eagle's
 J. Greiner, National Cancer Institute) and SK-N-SH human neuroblastoma cell
line (provided by L. Neckers, National Cancer Institute) were grown in Eagle's
minimum essential medium supplemented with 10% heat- inactivated fe line (provided by L. Neckers, National Cancer Institute) were grown in Eagle's minimum essential medium supplemented with 10% heat- inactivated fetal bovine serum, Eagle's minimum essential medium nonessential amino acids, minimum essential medium supplemented with 10% heat-inactivated fetal
bovine serum, Eagle's minimum essential medium nonessential amino acids,
20 mm HEPES, 2 mm glutamine, and penicillin-streptomycin. MCF-7 human
breast ca bovine serum, Eagle's minimum essential medium nonessential amino acids,
20 mm HEPES, 2 mm glutamine, and penicillin-streptomycin. MCF-7 human
breast cancer cell line was grown in improved minimal essential medium
suppleme 20 mm HEPES, 2 mm glutamine, and penicillin-streptomycin. MCF-7 human
breast cancer cell line was grown in improved minimal essential medium
supplemented with 10% heat-inactivated fetal bovine serum, 20 mm HEPES, 2
mm glut breast cancer cell line was grown in improved minimal essential medium
supplemented with 10% heat-inactivated fetal bovine serum, 20 mm HEPES, 2
mm glutamine, and penicillin-streptomycin. TMK-1 human gastric carcinoma
cell supplemented with 10% heat-inactivated fetal bovine serum, 2 mm glutamine, and penicillin-streptomycin. TMK-1 human g
cell line (provided by E. Tahara, Hiroshima University Scho
Hiroshima, Japan) was grown in RPMI 1640 wit

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Institutes of Health, Building 10, Room 5B38, Bethesda, MD 20892.

² The abbre ¹ To whom correspondence should be addressed, at National Cancer Institute, National
² The abbreviations used are: cAMP, cyclic AMP; R, regulatory (subunit); C, catalytic
(subunit); RI_G and RII_B, regulatory subunit Institutes or Health, Bullding 10, ROOM 3B38, Bethesda, MD 20

² The abbreviations used are: cAMP, cyclic AMP; Regulatory

(subunit); Rl_a and RII_B, regulatory subunits of cAMP-dependent

(type II, respectively; HEPES

SUPPRESSION OF MALIGNANCY BY AN ANTISENSE OLIGONUCLEOTIDE
For cell growth experiments, 2-3 × 10⁵ cells/60-mm dish were seeded, and
godeoxynucleotides were added at 4 h postseeding and then every 36 h RL, antisense S-oli SUPPRESSION OF MALIGNANCY BY AN ANTISENSE OLIGONUCLEOTIDE
For cell growth experiments, $2-3 \times 10^5$ cells/60-mm dish were seeded, and
oligodeoxynucleotides were added at 4 h postseeding and then every 36 h $R I_{\alpha}$ antise For cell growth experiments, $2-3 \times 10^5$ cells/60-mm dish were seeded, and oligodeoxynucleotides were added at 4 h postseeding and then every 36 h thereafter without changing medium. At desired times, cell counts in dupl For cell growth experiments, $2-3 \times 10^5$ cells/60-mm dish were seeded, and
oligodeoxynucleotides were added at 4 h postseeding and then every 36 h
thereafter without changing medium. At desired times, cell counts in dupl bligodeoxynucleotides
thereafter without chan
were performed on a 4
vesting cells with gent
blue dye exclusion.
Immunoprecipitati Immunoprecipitation of RI_a and RII_B cAMP Receptor Proteins after

Photoaffinity Labeling with 8-N₃-[³²P]cAMP. Cell extracts were prepared at

Photoaffinity Labeling with 8-N₃-[³²P]cAMP. Cell extracts were prepa

westing cells with gentle trypsinization. Cell viability was assessed by trypan
blue dye exclusion.
Immunoprecipitation of RI_a and RII_β cAMP Receptor Proteins after
Photoaffinity Labeling with 8-N₃-[³²P]cAMP. blue dye exclusion.

Immunoprecipitation of $\mathbb{R}I_{\alpha}$ and $\mathbb{R}II_{\beta}$ cAMP Receptor Proteins after

Photoaffinity Labeling with $8-N_3$ -[³²P]cAMP. Cell extracts were prepared at

0–4°C. The cell pellets (2 × 10° ce **Immunoprecipitation of RI_a and RII_B cAMP Receptor Proteins after
Photoaffinity Labeling with 8-N₃-[³²P]cAMP. Cell extracts were prepared a
0–4°C. The cell pellets (** 2×10^6 **cells), after 2 washes with phosphate Photoafflinity Labeling with 8-N₃-[³²P]cAMP.** Cell extracts were prepared at μ M concentration were more than 95% viable, but at 20 μ M concentration 0–4°C. The cell pellets (2 × 10⁶ cells), after 2 washes with aprotinin; and 0.5 mg/ml soybean trypsin inhibitor, filtered through a 0.45-um saline, were suspended in 0.5 ml buffer Ten (16) (20 mm Tris-HCl, pH 7.4; 0.1 in MaCl; 1% NP-40; 0.5% sodium deoxycholate; 5 mm MgCl₂; 0.1 mm pep-
statin; 0.1 mm antipain; 0.1 mm chymostatin; 0.2 mm leupeptin; 0.4 mg/ml M NaCl; 1% NP-40; 0.5% sodium deoxycholate; 5 mm MgCl₂; 0.1 mm pep-
statin; 0.1 mm antipain; 0.1 mm chymostatin; 0.2 mm leupeptin; 0.4 mg/ml
aprotinin; and 0.5 mg/ml soybean trypsin inhibitor, filtered through a 0.45- $\$ Eppendorf microfuge at 4° C. The supernatant was used as lysate. Protein statin; 0.1 mm antipain; 0.1 mm chymostatin; 0.2 mm leupeptin; 0.4 mg/ml den aprotinin; and 0.5 mg/ml soybean trypsin inhibitor, filtered throug aprotinin; and 0.5 mg/ml soybean trypsin inhibitor, filtered through a 0.45- μ m nen
pore size membrane), passed through a 20-gauge needle 5 times using a 1-ml exh
syringe, allowed to sit at 4°C for 15 min, and centrifug pore size membrane), passed through a 20-gauge needle 5 times using a 1-ml
syringe, allowed to sit at 4°C for 15 min, and centrifuged for 5 min in an
Eppendorf microfuge at 4°C. The supermatant was used as lysate. Protein syringe, allowed to sit at 4° C for 15 min, and centrifuged for 5 min in an
Eppendorf microfuge at 4° C. The supernatant was used as lysate. Protein ynu
concentration (usually between 1 and 5 mg/ml) was determined Eppendorf microfuge at 4° C. The supernatant was used as lysate. Protein ynuconcentration (usually between 1 and 5 mg/ml) was determined by the method be it labeling and immunoprecipitation of cAMP receptor proteins f concentration (usually between 1 and 5 mg/ml) was determined by the method of Lowry *et al.* (18) using bovine serum albumin as standard. Photoaffinity labeling and immunoprecipitation of cAMP receptor proteins followed th of Lowry *et al.* (18) using bovine serum albumin as standard. Photoaffinial
abeling and immunoprecipitation of cAMP receptor proteins followed the
method previously described (16). Anti-RI or anti-RII antiserum (provided Iabeling and immunoprecipitation of cAMP receptor proteins followed the method previously described (16). Anti-RI or anti-RII antiserum (provided by intersection). So Doskeland, University of Bergen, Bergen, Norway) and pr method previously described (16). Anti-RI or anti-RII antiserum (provided by
S. O. Doskeland, University of Bergen, Bergen, Norway) and protein A-
Sepharose were used for immunoprecipitation, the dissolved immunoprecipi-
t S. O. Doskeland, University of Bergen, Bergen, Norway) and Sepharose were used for immunoprecipitation, the dissolved imates were subjected to SDS-polyacrylamide gel electrophoresis solved proteins were electrotransferred Sharose were used for immunoprecipitation, the dissolved immunoprecipi-

Sharose were used for immunoprecipitation, the dissolved immunoprecipi-

Sharose were subjected to SDS-polyacrylamide gel electrophoresis, and the r tates were subjected to SDS-polyacrylamide gel electrophoresis, and the re-
solved proteins were electrotransferred to nitrocellulose sheets. Radioactivity
was detected by autoradiography using Kodak X-Omat film.
Poly(A)

Solved proteins were electrotransferred to introcellulose sheets. Radioactivity
solved proteins were electrotransferred to nitrocellulose sheets. Radioactivity
was detected by autoration. Poly(A)⁺ RNA was extracted from was detected by autoradiography using Kodak X-Omat film. we
 Poly(A)⁺ RNA Preparation. Poly(A)⁺ RNA was extracted from 2×10^7 go

cells for each treatment by QuickPrepTM mRNA Purification Kit (Pharmacia, ulg
 cells for each treatment by QuickPrepTM mRNA Purification Kit (Pharmacia, Piscataway, NJ), according to the manufacturer's instructions. Briefly, the cells were homogenized with 4 $\,\mathrm{M}$ guanidine thiocyanate and 0.5% Piscataway, NJ), according to the manufacturer's instructions. Briefly, the cells
were homogenized with 4 μ guanidine thiocyanate and 0.5% Sarkosyl
(N-lauroyl sarcosine/Na⁺) and then loaded onto an oligo (dThd) cellu Piscataway, NJ), according to the manufacturer's instructions. Briefly, the cells
were homogenized with 4 M guanidine thiocyanate and 0.5% Sarkosyl
(N-lauroyl sarcosine/Na⁺) and then loaded onto an oligo (dThd) cellulos (*N*-lauroyl sarcosine/Na⁺) and then loaded onto an oligo (dThd) cellulose span column. After washing 3 times with high salt buffer [10 mm Tris-HCl (pH 7.4), 1 mm EDTA, 0.5 m NaCl] and twice with low salt buffer [10 mm span column. After washing 3 times with high salt buffer $[10 \text{ m} \text{m} \text{ T} \text{r} \text{s} - \text{H} \text{C}]$
(pH 7.4), 1 mm EDTA, 0.5 m NaCl] and twice with low salt buffer $[10 \text{ m} \text{m} \text{ T} \text{r} \text{s} - \text{H} \text{C}]$ (pH 7.4), 1 mm EDT (pH 7.4), 1 mm ED
Tris-HCl (pH 7.4),
by washing with e
65°C. The RNA ob
tassium acetate.
Northern Blot A S-HCl (pH 7.4), 1 mm EDTA, 0.1 m NaCl], poly(A)⁺ RNA was recovered ^{Oligo} washing with elution buffer [10 mm Tris-HCl (pH 7.4), 1 mm EDTA] at show

Northern Blot Analysis. RNA pellets were suspended in 15 μ l denaturi by washing with elution buffer (10 mm Tris-HCl (pH 7.4), 1 mm EDTA] at shown 65°C. The RNA obtained was precipitated in ethanol with glycogen and possing acetate.
 Northern Blot Analysis. RNA pellets were suspended in 1

that
 Northern Blot Analysis. RNA pellets were suspended in 15 μ l denaturing pote

buffer (50% formamide-2.2 M formaldehyde-10 mm sodium phosphate, pH olig

7.5), incubated at 55°C for 15 min, and quenched on ice. Lo **Northern Blot Analysis.** RNA pellets were suspended in 15 μ l denaturing po
buffer (50% formamide-2.2 μ formaldehyde-10 mm sodium phosphate, pH
7.5), incubated at 55°C for 15 min, and quenched on ice. Loading buffer buffer (50% formamide-2.2 M formaldehyde-10 mM sodium phosphate, pH olism 7.5), incubated at 55°C for 15 min, and quenched on ice. Loading buffer (50% icid glycerol, 50% formamide, bromophenol blue; 7.5 µl) was added, and 7.5), incubated at 55°C for 15 min, and quenched on ice. Loading buffer (50% icity, as ce glycerol, 50% formamide, bromophenol blue; 7.5 µl) was added, and 10 µg antisense of mRNA were resolved on a 1.5% agarose gel conta mRNA were resolved on a 1.5% agarose gel containing 6.7% formaldehyde (v/v) and 20 mm sodium phosphate buffer, pH 6.8. The resolved RNA was blotted onto a Biotrans (ICN) nylon membrane and cross-linked by UV irradiation. buffer containing nick-translated cDNA probe. The one of the set of the solution. After prehybridization in 5 \times Denhardt's solution, 5 \times SSC, 50 mm sodium phosphate (pH 6.5), 0.1% SDS, 250 μ g/ml herring sperm DNA diation. After prehybridization in 5 × Denhardt's solution, 5 × SSC, 50 mm
sodium phosphate (pH 6.5), 0.1% SDS, 250 µg/ml herring sperm DNA, and
50% (v/v) formamide, the membrane was hybridized at 42°C in the same
buffer 50% (v/v) formamide, the membrane was hybridized at 42°C in the same buffer containing nick-translated cDNA probe (10⁷ cpm probe/ml hybridization solution). The same nitrocellulose filters were sequentially hybridized t buffer containing nick-translated cDNA probe (10⁷ cpm probe/ml hybridization solution). The same nitrocellulose filters were sequentially hybridized to the following ³²P-labeled probes: RI_{α} , 1.5 kilobase cDNA clon tion solution). The same nitrocellulose filters were sequentially hybridized to the following ³²P-labeled probes: RI_α , 1.5 kilobase cDNA clone, containing the entire coding region for the human RI_α (19) (pro the following ³²P-labeled probes: RI_a , 1.5 kilobase cDNA clone, containing the entire coding region for the human RI_a (19) (provided by T. Jahnsen, Institute of Pathology, Rikshospitalet, Oslo, Norway); C_a , 1.1 kb, entire coding region for the human $\overline{R}I_{\alpha}$ (19) (provided by T. Jahnsen, Institute
of Pathology, Rikshospitalet, Oslo, Norway); C_{α} , 1.1 kb, containing total
open-reading frame of human C_{α} cDNA (20) (provid open-reading frame of human C_{α} cDNA (20) (provided by S. K. Hanks, The Salk Institute, San Diego, CA); and human β -actin (Oncor p7000 β -actin). The membrane was washed 4 times in 2 × SSC and 0.1% SDS at room te for 5 min followed by 2 washes using $0.1 \times$ SSC and 0.1% SDS at 50°C.
Autoradiography was performed using XAR2 (Kodak) film.
RESULTS AND DISCUSSION

Nuclease resistance is one of the major factors to be concerned with
in the use of oligodeoxynucleotides for the antisense inhibition of
gene expression. The normal unmodified β -oligodeoxynucleotides are **INTERT AND DISCUSSION**
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gene expression. The normal unmodified β -oligodeoxynucleo **RESULTS AND DISCUSSION**
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gene expression. The normal unmodified β -oligodeoxynucle **RESULTS AND DISCUSSION**
Nuclease resistance is one of the major factors to be concerned wi
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gene expression. The normal unmodified β -oligodeoxynucleotide Nuclease resistance is one of the major factors to be concerned with
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gene expression. The normal unmodified β -oligodeoxynucleotides are
far more sensi The use of oligodeoxynucleotides for the antisense inhibition of the use of oligodeoxynucleotides for the antisense inhibition of the are expression. The normal unmodified β -oligodeoxynucleotides a far more sensitive t given expression. The normal unmodified β -oligodeoxynucleotides are
far more sensitive to nuclease hydrolysis than are α -oligodeoxynu-
cleotides, methylphosphonate, or phosphorothioate oligodeoxynucle-
otides (14, 2 Far more sensitive to nuclease hydrolysis than are α -oligodeoxynucleotides, methylphosphonate, or phosphorothioate oligodeoxynucleotides (14, 21, 22). In this study, we have chosen S-oligodeoxynucleotide to block the e cleotides, methylphosphonate, or phosphorothioate oligodeoxynucle-
otides (14, 21, 22). In this study, we have chosen S-oligodeoxynucle-
otide on the growth inhibition of LS-1747 colon carcinoma cells. Cells were exposed otides $(14, 21, 22)$. In this study, we have chosen S-oligodeoxynucle-

were performed on a Coulter counter (Coulter Corp., Hialeah, FL) after har-
concentration inducing 50% inhibition of cell proliferation of RI_a LS-174T human colon carcinoma cells were exposed for 6 days to R ANTISENSE OLIGONUCLEOTIDE

LS-174T human colon carcinoma cells were exposed for 6 days to

RI_α antisense S-oligodeoxynucleotide at different concentrations, and

cell growth was compared with untreated cells. As shown EXTREE CONSIDENT UNIVERSITY ANTISENSE OLIGONUCLEOTIDE

LS-174T human colon carcinoma cells were exposed for 6 days to

RI_q antisense S-oligodeoxynucleotide at different concentrations, and

cell growth was compared with LS-174T human colon carcinoma cells were exposed for 6 days to RI_{α} antisense S-oligodeoxynucleotide at different concentrations, and cell growth was compared with untreated cells. As shown in Fig. 1, the concentration LS-174T human colon carcinoma cells were exposed for 6 days to RI_{α} antisense S-oligodeoxynucleotide at different concentrations, and cell growth was compared with untreated cells. As shown in Fig. 1, the concentration RI_{α} antisense S-oligodeoxynucleotide at different concentrations, and
cell growth was compared with untreated cells. As shown in Fig. 1, the
concentration inducing 50% inhibition of cell proliferation of RI_{α}
ant cell growth was compared with untreated cells. As shown in Fig. 1, the concentration inducing 50% inhibition of cell proliferation of RI_{α} antisense S-oligodeoxynucleotide was 6.0 μ M. Trypan blue dye exclusion test concentration inducing 50% inhibition of cell proliferation of RI, antisense S-oligodeoxynucleotide was 6.0 μ M. Trypan blue dye exclusion test showed that cells exposed to RI_a antisense oligomer up to 10 μ M concen antisense S-oligodeoxynucleotide was 6.0 μ M. Trypan blue dye exclusion test showed that cells exposed to RI_{α} antisense oligomer up to 10 μ M concentration were more than 95% viable, but at 20 μ M concentration Soligodeoxynucleotide vias of pair. Figure only of the sign and sign test showed that cells exposed to RI_{α} antisense oligomer up to 10 pm concentration only 70% of cells were viable, indicating nonspecific cytotoxicit μ m concentration were more than 95% viable, but at 20 μ m concentration only 70% of cells were viable, indicating nonspecific cytotoxicity of the oligonucleotide at higher concentrations. RI_α antisense S-oligodeoxy tration only 70% of cells were viable, indicating nonspecific cytotox-
icity of the oligonucleotide at higher concentrations. RI_{α} antisense
S-oligodeoxynucleotide induced growth inhibition in a time-depen-
dent manner exhibited a reduced growth inhibition in a time-dependent manner (Fig. 2). While untreated cells demonstrated an exponential rate of growth, cells exposed to 6 μ m RI_{α} antisense oligomer exhibited a reduced growth S-oligodeoxynucleotide induced growth inhibition in a time-dependent manner (Fig. 2). While untreated cells demonstrated an exponential rate of growth, cells exposed to 6 μ m RI_a antisense oligomer exhibited a reduced dent manner (Fig. 2). While untreated cells demonstrated an exponential rate of growth, cells exposed to 6 μ m RI_{α} antisense oligomer exhibited a reduced growth rate within 4-5 days and eventually stopped proliferat be in the range of 7-19 h (22), and the RI_α antisense oligomer
schibited a reduced growth rate within 4-5 days and eventually
stopped proliferating by day 6. Since the half-lives of a S-oligodeox-
ynucleotide in various exhibited a reduced growth rate within 4-5 days and eventually
stopped proliferating by day 6. Since the half-lives of a S-oligodeox-
ynucleotide in various biological fluids and sera have been shown to
be in the range of stopped proliferating by day 6. Since the half-lives of a S-oligodeox-
ynucleotide in various biological fluids and sera have been shown to
be in the range of 7–19 h (22), and the RI_{α} antisense oligonucleotide
was ynucleotide in various biological fluids and sera have been shown to
be in the range of 7–19 h (22), and the RI_{α} antisense oligonucleotide
was added to the cell culture every 36 h, it is unlikely that the growth
inhib be in the range of 7–19 h (22), and the RI_{α} antisense oligonucleotide was added to the cell culture every 36 h, it is unlikely that the growth inhibition observed (Fig. 2) was due to a nonspecific cytotoxicity caused was added to the cell culture every 36 h, it is unlikely that the growth inhibition observed (Fig. 2) was due to a nonspecific cytotoxicity caused by the accumulation of the oligomer. Treatment with mismatched (random) se inhibition observed (Fig. 2) was due to a nonspecific cytotoxicity caused by the accumulation of the oligomer. Treatment with mis-
matched (random) sequence S-oligodeoxynucleotide (6 μ w) had no
appreciable effect on ce minimum osset via (1 ig. 2) was due to a monspective cytoconomy
caused by the accumulation of the oligomer. Treatment with mis-
matched (random) sequence S-oligodeoxynucleotide (6 μ m) had no
appreciable effect on cell matched (random) sequence S-oligodeoxynucleotide (6 μ m) had no
appreciable effect on cell growth (Fig. 2). Interestingly, the growth
inhibitory effect of RI_a antisense oligomer was abolished when cells
were exposed s appreciable effect on cell growth (Fig. 2
inhibitory effect on cell growth (Fig. 2
inhibitory effect of RI_{α} antisense oligome
were exposed simultaneously to both RI_{g}
godeoxynucleotides; RI_{Ig} antisense oligor
ula inity interpretent of RI_{α} antisense oligomer was abolished when cells
ree exposed simultaneously to both RI_{α} and RII_B antisense S-oli-
deoxynucleotides; RII_B antisense oligomer alone had a slight stim-
tory unified and S-oligodeoxynucleotides; RII_B antisense oligomer alone had a slight stimulatory effect on cell growth (Fig. 2).
We next examined the effect of RI_{α} antisense oligodeoxynucleotide unmodified and S-oligodeo

65°C. The RNA obtained was precipitated in ethanol with glycogen and po-
tassium acetate.
that caused by 6 μ M S-oligodeoxynucleotide, demonstrating a greater
Northern Blot Analysis. RNA pellets were suspended in 15 mere exposed simulations, if your Riquin High antisense of on
godeoxynucleotides; RII_B antisense oligomer alone had a slight stim-
ulatory effect on cell growth (Fig. 2).
We next examined the effect of RI_α anti ulatory effect on cell growth (Fig. 2).

We next examined the effect of RI_{α} antisense oligodeoxynucleotide

unmodified and S-oligodeoxynucleotide on the growth of other human

cancer cell lines, including SK-N-SH neur We next examined the effect of RI_{α} antisense oligodeoxynucleotide
unmodified and S-oligodeoxynucleotide on the growth of other human
cancer cell lines, including SK-N-SH neuroblastoma, MCF-7 breast,
and TMK-1 gastric We next examined the effect of M_{α} annisome ongedeely indetective
unmodified and S-oligodeoxynucleotide on the growth of other human
cancer cell lines, including SK-N-SH neuroblastoma, MCF-7 breast,
and TMK-1 gastric cancer cell lines, including SK-N-SH neuroblastoma, MCF-7 breast, and TMK-1 gastric carcinoma cells. As shown in Fig. 3, RI_{α} antisense oligomer induced growth inhibition of these cells as potently as that shown in and TMK-1 gastric carcinoma cells. As shown in Fig. 3, RI_{α} antisense oligomer induced growth inhibition of these cells as potently as that shown in LS-174T cells. Fig. 3 also shows that the unmodified antisense oli oligomer induced growth inhibition of these cells as potently as that shown in LS-174T cells. Fig. 3 also shows that the unmodified antisense oligomer at 30 μM produced a growth inhibitory effect similar to that caused by shown in LS-174T cells. Fig. 3 also shows that the unmodified anti-
sense oligomer at 30 μ M produced a growth inhibitory effect similar to
that caused by 6 μ M S-oligodeoxynucleotide, demonstrating a greater
potency sense oligomer at 30 μ M produced a growth inhibitory effect similar to that caused by 6 μ M S-oligodeoxynucleotide, demonstrating a greater potency of phosphorothioate oligomer as compared with unmodified oligomer. T that caused by 6 μ M S-oligodeoxynucleotide, demonstrating a greater potency of phosphorothioate oligomer as compared with unmodified oligomer. The growth inhibition was not due to nonspecific cytotoxicity, as cells wer potency of phosphorothioate oligomer as compared with unmodified oligomer. The growth inhibition was not due to nonspecific cytotoxicity, as cells were more than 90% viable after exposure to RI_{α} antisense oligomer for

Fig. 1. Concentration dependency of \mathbb{R}^1 antisense phosphorothioate oligodeoxynucle-
otide on the growth inhibition of LS-174T colon carcinoma cells. Cells were exposed for
6 days with \mathbb{R}^1 antisense oligomer Fig. 1. Concentration dependency of RI_a antisense phosphorothioate oligodeoxynucle-
otide on the growth inhibition of LS-174T colon carcinoma cells. Cells were exposed for
6 days with RL_{a} antisense oligomer at var experiments.

SUPPRESSION OF MALIGNANCY BY AN ANTISENSE OLIGONUCLEOTIDE
concentrations, respectively, had no growth inhibitory effect up to 7 **3**
LS-174T concentrations, respectively, had no growth inhibitory effect up to 7
days of exposure (data not shown).
The growth inhibition induced by RI_{α} antisense oligodeoxynucle-

SUPPRESSION OF MALIGNANCY

notination induced by RI_{α} antisense oligodeoxynucle

de induced a change in cell morphology. SK-N-SH neuroblastom

de induced a change in cell morphology. SK-N-SH neuroblastom suppression of MALIGNANCY BY AN /
concentrations, respectively, had no growth inhibitory effect up to 7
days of exposure (data not shown).
The growth inhibition induced by RI_{α} antisense oligodeoxynucle-
otide induced concentrations, respectively, had no growth inhibitory effect up to 7
days of exposure (data not shown).
The growth inhibition induced by RI_{α} antisense oligodeoxynucle-
otide induced a change in cell morphology. SK-Ndays of exposure (data not shown).

The growth inhibition induced by RI_{α} antisense oligodeoxynucle-

otide induced a change in cell morphology. SK-N-SH neuroblastoma

cells exposed to 6 μ M RI_{α} antisense S-oligo The growth inhibition induced by RI_{α} antisense oligodeoxynucle-
otide induced a change in cell morphology. SK-N-SH neuroblastoma
cells exposed to 6 μ M RI_{α} antisense S-oligodeoxynucleotide for 6 days
exhibited a otide induced a change in cell morphology. SK-N-SH neuroblastoma
cells exposed to 6 μ M RI_{α} antisense S-oligodeoxynucleotide for 6 days
exhibited a striking change in morphology: cells became flat, exhib-
ting extr cells exposed to 6 μ m RI_{α} antisense S-oligodeoxynucleotide for 6 days
exhibited a striking change in morphology: cells became flat, exhib-
iting extremely enlarged cytoplasm (Fig. 4). In the case of HL-60
cells, t exhibited a striking chain
iting extremely enlarge
cells, the RI_{α} antisense
4-5 days elicited a mord
differentiation (15).
To provide evidence

Example a standary emails in increasingly: cells occulate talt, calibriting extremely enlarged cytoplasm (Fig. 4). In the case of HL-60 cells, the RI_α antisense oligodeoxynucleotide (15 μm) treatment for 4–5 days elicit cells, the RI_α antisense oligodeoxynucleotide (15 μm) treatment for
4–5 days elicited a monocytic morphological change, indicating cell
differentiation (15).
To provide evidence that the growth inhibition induced in cel 4–5 days elicited a monocytic morphological change, indicating cell
differentiation (15). To provide evidence that the growth inhibition induced in cells
exposed to RI_{α} antisense oligodeoxynucleotide was directly rel shown in Fig. 5, the human RI_{α} cDNA probe detected a major 4.4-
kilobase mRNA (23) in growing, untreated LS-174T cells (Fig. 5, RI_{α}, CDNA probe detected a major 4.4-
kilobase mRNA (23) in growing, untreated LS-1 To provide evidence that the growth inhibition induced in cells
exposed to RI_{α} antisense oligodeoxynucleotide was directly related to
the arrest of RI_{α} gene expression, we measured RI_{α} mRNA level. As
shown **Langthanuary and S-oligodeoxynucleotide was directly related to**
the arrest of RI_α gene expression, we measured RI_α mRNA level. As
shown in Fig. 5, the human RI_α cDNA probe detected a major 4.4-
kilobase mRNA (23) the arrest of \overline{RI}_{α} gene expression, we measured \overline{RI}_{α} mRNA level. As
shown in Fig. 5, the human \overline{RI}_{α} cDNA probe detected a major 4.4-
kilobase mRNA (23) in growing, untreated LS-174T cells (Fig. 5, \over shown in Fig. 5, the human RI_{α} cDNA probe detected a major 4.4-
kilobase mRNA (23) in growing, untreated LS-174T cells (Fig. 5, RI_{α}, E
Lane 4). Treatment with RI_{α} antisense S-oligodeoxynucleotide (6 μ M)
 kilobase mRNA (23) in growing, untreated LS-174T cells (Fig. 5, RL_a, E
Lane 4). Treatment with RL_a antisense S-oligodeoxynucleotide (6 μ m)
for 6 days resulted in almost complete blockage in the RL_a gene
expressio Lane 4). Treatment with RI_{α} antisense S-oligodeoxynucleotide (6 μ M)
for 6 days resulted in almost complete blockage in the RI_{α} gene
expression (Fig. 5, RI_{α} , Lane 1); the steady state level of $\text{RI$ for 6 days resulted in almost complete blockage in the RI_{α} gene
expression (Fig. 5, RI_{α} , *Lane 1*); the steady state level of RI_{α} mRNA
decreased to 10% that of control cells (Fig. 5). Previous studies have
sho expression (Fig. 5, RI_a, *Lane 1*); the steady state level of RI_a mRNA
decreased to 10% that of control cells (Fig. 5). Previous studies have
shown (24) that LS-174T cells contain mainly type I protein kinase,
and thu suppression of C expression. In fact, C_{α} mRNA level decreased, in oxynucleotide (\Box) or its phosphorothioate analog (Δ) at indicated doses (see "Materials"). At indicated times, cell counts in duplicate were per shown (24) that LS-174T cells contain mainly type I protein kinase,
and thus RI_a is the major, if not only, cAMP receptor in these cells. As
cells express R and C in a 1:1 ratio (25), it was expected that RI_a
suppress and thus RI_{α} is the major, if not only, cAMP receptor in these cells. As
cells express R and C in a 1:1 ratio (25), it was expected that RI_{α}
antisense oligomer, which blocks RI_{α} expression, would also resul and thus M_{α} is the impot, it not only, evident receptor in these cents. The cells express R and C in a 1:1 ratio (25), it was expected that RI_{α} antisense oligomer, which blocks RI_{α} expression, would also re exposure of cells to RI_{IG} to RI_{IG} expression, would also result in equipments of C expression. In fact, C_a mRNA level decreased, in parallel with the decrease in RI_a mRNA in cells exposed to RI_a sending antisens suppression of C expression. In fact, C_{α} mRNA level decreased, in a parallel with the decrease in RI_{α} mRNA in cells exposed to RI $_{\alpha}$ santisense S-oligodeoxynucleotide (Fig. 5, C_{α} , Lane 1). In contrast, e parallel with the decrease in RI_{α} mRNA in cells exposed to RI $_{\alpha}$ and antisense S-oligodeoxynucleotide (Fig. 5, C_{α}, *Lane 1*). In contrast, exposure of cells to RI $_{\beta}$ antisense S-oligodeoxynucleotide (6 μ parallel with the decrease in Rl_α mRNA in cells exposed to Rl_α sent the average values \pm SD of three separate experiments.

antisense S-oligodeoxynucleotide (Fig. 5, C_α, Lane 1). In contrast,

exposure of cells t exposure of cells to RII_B antisense S-oligodeoxynucl
6 days), which led to a slight stimulation of cell β
brought about an effect on RI_a and C_a mRNA oppinduced by RI_a antisense oligomer: cells exhibited a
press It has been shown (13, 26) that the striking growth-inhibitory effect site-selective 8-Cl-cAMP demonstrated in cancer cells correlates with the striking growth-inhibitory effect site-selective 8-Cl-cAMP demonstrated in ca of days), which icd to a slight simulation of een growth (Fig. 2), of brought about an effect on RI_{α} and C_{α} mRNA opposite from that induced by RI_{α} antisense oligomer: cells exhibited an enhanced expr

mateca by \overline{RI}_{α} and \overline{CI}_{α} mRNA (Fig. 5, *Lane 2)*.

It has been shown (13, 26) that the striking growth-inhibitory effect

of site-selective 8-Cl-cAMP demonstrated in cancer cells correlates

with its selectiv It has been shown (13, 26) that the striking growth-inhibitory effect
of site-selective 8-Cl-cAMP demonstrated in cancer cells correlates
with its selective modulation of two types of cAMP receptor proteins:
a marked down of site-selective 8-Cl-cAMP demonstrated in cancer cells correlates
with its selective modulation of two types of cAMP receptor proteins:
a marked down-regulation of RI_{α} receptor; and an up-regulation of M_r
RII<sub> β with its selective over-example and on two types of cAMP receptor proteins:
a marked down-regulation of RI_{α} receptor; and an up-regulation of RI_{β} receptor. Present results show that the effect of RI_{α} antisense

TIME (days) in

Fig. 2. Time-dependent growth inhibitory effect of RI_a antisense S-oligodeoxynucle-

otide on LS-174T colon carcinoma cells. Cells were untreated (O) or treated with 6 μ antisense

S-oligodeoxynucle **HME (Cays)**

Fig. 2. Time-dependent growth inhibitory effect of Rl_a antisense S-oligodeoxynucle-

or treated (O) or treated times, cell counts in duplicate (Rl_a antisense (O), $6 \mu M$ Rl_a antisense (B), $6 \mu M$ Rl_a Fig. 2. Time-dependent growto
tide on LS-174T colon carcinotic of RI_a and
S-oligodeoxynucleotide of RI_a and
+ 6 μ M RII_B antisense (O), or
times, cell counts in duplicate we
of three separate experiments.

Time (Days)
Fig. 3. Effect of \mathbb{R}^1 antisense oligodeoxynucleotide on the growth of human cancer
cell lines. Cells were grown in the absence (\bigcirc) and presence of \mathbb{R}^1 antisense oligode-
oxynucleotide (\Box Fig. 3. Effect of RI_a antisense oligodeoxynucleotide on the cell lines. Cells were grown in the absence (\bigcirc) and presence coxynucleotide (\bigcirc) or its phosphorothioate analog (Δ) at indicated and Methods"). At in cell lines. Cells were grown in the absence (O) and presence of RI_a antisense oligode-
oxynucleotide (\square) or its phosphorothioate analog (Δ) at indicated doses (see "Materials
and Methods"). At indicated times, cel

oxynucleotide (\square) or its phosphorothioate analog (Δ) at indicated doses (see "Materials
and Methods"). At indicated times, cell counts in duplicate were performed. Data repre-
sent the average values \pm SD of thre Suppose the average values \pm SD of the average values \pm SD of the antisense oligonucle fairly constant (Fig. 5).
We next examined the effect of $\frac{1}{2}$ we next examined the effect of $\frac{1}{2}$ we next examined the ese changing levels of RI_{α} and C_{α} mRNA were the specific effect
the antisense oligonucleotides, as β -actin mRNA level remained
rly constant (Fig. 5).
We next examined the effect of the RI_{α} antisense oligon of the antisense oligonucleotides, as β -actin mRNA level remained

induced by RI_{α} antisense oligomer: cells exhibited an enhanced ex-

we next examined the effect of the RI_{α} antisense oligonucleotide on

the selective modulation (13, 26) that the striking growth-inhibitory effe Fig. 2. Time-dependent growth inhibitory effect of RI_a antisense S-oligodeoxynucle
or Let antisense S-oligodeoxynucle
or Let antisense S-oligodeoxynucle
or Let antisense S-oligodeoxynucle
or Let antisense S-oligodeoxynu These changing levels of RI_{α} and C_{α} mRNA were the specific effect
of the antisense oligonucleotides, as β -actin mRNA level remained
fairly constant (Fig. 5).
We next examined the effect of the RI_{α} antise fairly constant (Fig. 5).

We next examined the effect of the RI_{α} antisense oligonucleotide of

the levels of RI_{α} and RI_{β} proteins in these cells using photoaffinity

labeling with $8-N_3-[^{32}P]$ cAMP followed We next examined the effect of the RI_{α} antisense oligonucleotide on
the levels of RI_{α} and RI_{β} proteins in these cells using photoaffinity
labeling with 8-N₃-[³²P] cAMP followed by immunoprecipitation
with the levels of RI_{α} and RI_{β} proteins in these cells using photoaffinity
labeling with $8-N_3-[3^2P]$ cAMP followed by immunoprecipitation
with anti-RI and -RII antisera and SDS-polyacrylamide gel electro-
phoresis. In labeling with 8-N₃-[³²P] cAMP followed by immunoprecipitation
with anti-RI and -RII antisera and SDS-polyacrylamide gel electro-
phoresis. In control LS-174T cells, the anti-RI antibody detected the
 M_r 48,000 protei with anti-RI and -RII antisera and SDS-polyacrylamide gel electroone species of RII, the M_r 52,000 protein, which did not comigrate with the M_r 54,000 bovine heart RII_a, that we refer to as RII_B M_r 48,000 protein, which comigrated with rabbit skeletal muscle RI_a, that we refer to as RI_a (Fig. 6A), while the anti-RII antibody, which cross-reacts with both RII_a and RII_B in human cells³, detected only on (m_r -6,000 plotent, which configured with rabon sected mascle $\kappa_{1\alpha}$,
that we refer to as RI_{α} (Fig. 6A), while the anti-RII antibody, which
cross-reacts with both RI_{α} and RI_{β} in human cells³, that we felct to as \mathbf{kl}_{α} (Fig. 04), while the anti-Kri antibody, which
cross-reacts with both \mathbf{RI}_{α} and \mathbf{RI}_{B} in human cells³, detected only
one species of RII, the M_r 52,000 protein, which did not c one species of RII, the M_r 52,000 protein, which did not comigrate
with the M_r 54,000 bovine heart RII_a, that we refer to as RII_B
(Fig. 6B). That the anti-RII-antibody failed to detect RII_a, which
exhibits M_r with the M_r 54,000 bovine heart RII_a, that we refer to as RII_B (Fig. 6B). That the anti-RII-antibody failed to detect RII_a, which exhibits M_r 50,000–51,000 in human cells (9, 10, 27–30) and can be phosphorylated which the M_f 54,000 bovine heart Kin_a, that we feld to as King
(Fig. 6B). That the anti-RII-antibody failed to detect RII_a, which
exhibits M_f 50,000–51,000 in human cells (9, 10, 27–30) and can be
phosphorylated t exhibits M_r , 50,000–51,000 in human cells (9, 10, 27–30) and can be phosphorylated to become M_r , 54,000 (30), indicates that these cells contained RII_{α} at a low undetectable level. Treatment with RI_{α} antisen phosphorylated to become M_r 54,000 (30), indicates that these cells
contained RII_{α} at a low undetectable level. Treatment with RI_{α} anti-
sense S-oligodeoxynucleotide brought about a marked reduction in
RI_{α} contained RII_α at a low undetectable level. Treatment with RI_α antisense S-oligodeoxynucleotide brought about a marked reduction in RI_α level (Fig. 6A, *Lane 2*) with an increase in RII_β level (Fig. 6B, *Lane 2*) Sense S-oligodeoxynucleotide brought about a marked reduction in RI_{α} level (Fig. 6A, *Lane 2*) with an increase in RI_{β} level (Fig. 6B, *Lane 2*) as compared with untreated control cells. 8-Cl-cAMP treatment RI_{α} level (Fig. 6A, *Lane 2*) with an increase in RII_B level (Fig. 6B, *Lane 2*) as compared with untreated control cells. 8-Cl-cAMP treatment brought about an effect on RI_α and RII_B levels similar to that of Lane 2) as compared with untreated control cells. 8-CI-cAMP treat-
ment brought about an effect on RI_{α} and RII_{β} levels similar to that of RI_{α} antisense oligomer (Fig. 6A, B, Lane 4), whereas RII_{β} antisense Earte 2) as compared with unitedate control cents. 6-CF-CHMI treatment brought about an effect on RI_{α} and RI_{β} levels similar to that of RI_{α} antisense oligomer (Fig. 6A, B, Lane 4), whereas RI_{β} RI_{α} antisense oligomer (Fig. 6A, *B*, *Lane 4*), whereas RII_B antisense S-oligodeoxynucleotide brought about effects opposite those of RI_{α} antisense oligomer (Fig. 6,A and *B*, *Lane 3*) (16). The marked reduc S-oligodeoxynucleotide brought about effects opposite those of RI_{α}
S-oligodeoxynucleotide brought about effects opposite those of RI_{α}
antisense oligomer (Fig. 6,A and B, Lane 3) (16). The marked reduc-
tion antisense oligomer (Fig. 6,A and B, Lane 3) (16). The marked reduction in the band detected with the anti-RII antibody (Fig. 6B, Lane 3) in cells treated with RII_B antisense oligonucleotide further supports that the ant tion in the band detected with the anti-RII antibody (Fig. 6B, Lane 3) in cells treated with RII_B antisense oligonucleotide further supports that the anti-RII antibody detected RII_B but not RII_a in LS-174T cells. Th that the stated with RII_B antisense oligonucleotide further supports
that the anti-RII antibody detected RII_B but not RII_α in LS-174T cells.
These results show that RI_α and RII_B antisense oligod in complement of the anti-RII antibody detected RII_B but not RII_a in LS-174T cells.
These results show that RI_a and RII_B antisense oligodeoxynucleotides
each exerted a specific effect on their respective target mR These results show that RI_{α} and RII_{β} antisense oligodeoxynucleotides
each exerted a specific effect on their respective target mRNAs and
that their targeting of either isoform of R subunit expression resulted
in c These results show that Ki_a and Ki_B antiselise ongodeoxynacteondes
each exerted a specific effect on their respective target mRNAs and
that their targeting of either isoform of R subunit expression resulted
in compens that their targeting of either isoform of R subunit expression resulted
in compensatory enhancement of the expression of the other form of
R subunit. The arrest of RL_{α} gene expression was commonly observed
in SK-Nin compensatory enhancement of the expression of the other form of R subunit. The arrest of RI_{α} gene expression was commonly observed in SK-N-SH (Table 1), MCF-7 and TMK-1 (data not shown) cancer cells, those that exh Blocked from the expression was commonly observed
in SK-N-SH (Table 1), MCF-7 and TMK-1 (data not shown) cancer
cells, those that exhibited growth arrest (Fig. 3) after exposure to RI_α
antisense oligonucleotide. Importa Expansion of both RI_o

Sisense oligonucleotide. Importantly,

Cho-Chung et al., unpublished observation.

Fig. 4. Change in morphology of SK-N-SH neuroblastoma cells treated with RI_a antisense S-oligodeoxynucleotide. Cells untreated (control) or treated for 6 days with RI_a antisense oligomer, 6 µM, or mismatched sequence oligomer, 6 µM (random), were seeded at 10⁴ cells/ml onto 4-chamber tissue culture slides and grown for 48 h, ± continued treatment with the oligomers. Cells were fixed and stained by Wright's stain \times 120.

Fig. 5. Decreased RI_{α} and C_{α} mRNA levels in LS-174T colon carcinoma cells exposed to RI_{α} antisense oligodeoxynucleotide. Cells were treated for 6 days with RI_{α} antisense S-oligodeoxynucleotide and RII_B antisense S-oligodeoxynucleotide each at 6 um concentration (see "Materials and Methods") or with 8-Cl-cAMP (10 µм) for 6 days (single treatment at 5 h after seeding). Poly(A)⁺ RNA was isolated from control (untreated) and treated cells, and Northern blot analysis was performed as described in "Materials and Methods." Lane 1, cells exposed to RI_o antisense oligomer; Lane 2, cells exposed to RII_o antisense oligomer; Lane 3, cells treated with 8-Cl-cAMP; Lane 4, untreated control cells. The same nitrocellulose filters were sequentially hybridized to the ³²P-labeled nicktranslated cDNA probes (see "Materials and Methods"). The data in the table represent quantification by densitometric scanning of the autoradiograms. The data are expressed relative to the levels in control cells, which are set equal to 1 arbitrary unit. The data represent an average value of three separate experiments.

of cells to both RI_{α} and RI_{β} antisense oligodeoxynucleotides simultaneously, cells were no longer growth inhibited (Table 1), demonstrating a clear reciprocal relationship between RI_{α} and RII_{β} expression in growth control of these cells.

In LS-174T cells that contained mainly RI_{α} , RI_{α} antisense oligonucleotide treatment resulted in the suppression of both RI_{α} and C_{α} subunits. Thus, growth inhibition induced by RI_{α} antisense oligomer in LS-174T cells, at least in part, could be due to down-regulation of C_{α} subunit. However, no direct role of C subunit of protein kinase has been shown (15) in the differentiation induced by RI_{α} antisense oligonucleotide in HL-60 leukemia cells that contained both RI_{α} and RII_{α} . Suppression of RI_{α} by RI_{α} antisense oligodeoxynucleotide, which brought about a compensatory increase in RII_B, induced growth inhibition and differentiation in HL-60 cells in which C subunit expression was not down-regulated and remained the same. Differentiation of HL-60 cells was abolished when cells were exposed to both RI_{α} and RI_{α} antisense oligomers, eliminating both RI_{α} and RI_{α} . However, differentiation was not blocked when RI_{α} and RII_{α} were eliminated by RI_{α} and RII_{α} antisense oligomers. A situation similar to exposure of cells to both RI_{α} and RII_{β} antisense oligodeoxynucleotides was demonstrated by overexpression of RII_{α} in ras-transformed NIH3T3 cells in which RI_{α} and RI_{β} were reduced, but did not produce growth inhibition or reverse transformation (31, 32). Thus, the RI_{α} antisense oligonucleotide induced growth inhibition with or without C_{α} suppression. The common effect of RI_{α} antisense oligonucleotide was, however, down-regulation of RI_{α} with up-regulation of $RII₆$.

As RII_{α} forms the more favored complex with the C subunit as compared with other R subunits $(4, 32)$, it may serve as a reservoir to

Fig. 6. Effect of RI_{α} antisense oligodeoxynucleotide on the levels of RI_{α} and RI_{β} cAMP receptor proteins in LS-174T colon carcinoma cells. Cells were treated with RI_a or RII_B antisense S-oligodeoxynucleotide or with 8-CI-cAMP as described in Fig. 5. Preparation of cell extracts, the photoaffinity labeling with 8-N₃-[³²P]cAMP, immunoprecipitation using the anti-RI or anti-RII antiserum and protein A-Sepharose, and the following SDS-polyacrylamide gel electrophoresis were performed as described in "Materials and Methods." Preimmune serum controls were performed simultaneously, and no immunoprecipitated band was detected. $RI\alpha$, the M_r 48,000 RI (Sigma); $RII\alpha$, the M_r 56,000 RII (Sigma). Lanes R/α and R/α are from photoaffinity labeling only; A and B, Lanes 1-4, photoaffinity labeling followed by immunoprecipitation with anti-RI and anti-RII antisera, respectively. The data in the table represent quantification by densitometric scanning of the autoradiograms. The data are expressed relative to the levels in control (untreated) cells, which are set equal to 1 arbitrary unit. The data represent an average value of three experiments.

 0.2

 2.5

8-CI-cAMP

4

SUPPRESSION OF MALIGNANCY BY AN ANTISENSE OLIGONUCLEOTIDE
Table 1 Effect of Rl_a and Rll_ig antisense S-oligodeoxynucleotides added singly or in Rep., 7: 49–60, 1983.
combination on the growth and levels of Rl_a and Rl *combination on the growth and levels of RI_a and RII_B antisense S-oligodeoxynucleotides added singly or in
combination on the growth and levels of RI_a and RII_B in SK-N-SH neuroblastoma
cells cells* Table 1 *Effect of Rl_a and Rll_B antisense S-oligodeoxynucleotides added singly or in*

combination on the growth and levels of RI_a and Rll_B in SK-N-SH neuroblastoma

Cells were treated with RI_a or RI_B antisense S

Figure 1 Eyect of KL_a and KL_B anisense S-otrgoadeoxynucteonaes dadea singly or in
combination on the growth and levels of RL_a and RL_B in SK-N-SH neuroblastoma
Cells were treated with RL_a or Rll_B antisense S-oligodeo Cells were treated with \mathbb{R}^1_{α} or \mathbb{R}^1_{β} antiserse S-oligodeoxynucleotide, singly or in
combination (see "Materials and Methods"). On day 5, preparation of cell extracts,
photoaffinity labeling with 8-N₃ Let the treated with Kl_a or Kit_l, and Senset S-otregode oxynucteduce, singly or it combination (see "Materials and Methods"). On day 5, preparation of cell extracts in the autority labeling with $8-N_3$ -[³²P]cAMP, im combination (see "Materials and Methods"). On day 5, preparation of cell extracts,

photoaffinity labeling with 8-N₃-[³²P]cAMP, immunoprecipitation using the anti-RI or trations and distribution of cyclic nucleotide-d photoarinny labeling with 6-193-1 PJCAMP, immunippediploation using the anti-K1 or
the fill antiserum and protein A-Sepharose, and the following SDS-polyacrylamide gel
electrophoresis were performed as described in "Materi represent an average value of three separates, and une following SDS-polyacrylaming general and RII_B levels represent quantification by densitometric scanning of the autoradiograms of SDS-polyacrylamide gel electrophores $\mathbf{R}_{1\alpha}$ and $\mathbf{R}_{1\beta}$ revers represent quantinedator by densitioned is same expressed relative to obtained by comparison of SDS-polyarcylamide gel electrophoresis. The data represent an average value of three sepa the levels in control (untreated) cells, which are set equal to 1 arbitrary unit. The data represent an average value of three separate experiments. On day 5, cell counts in duplicate were performed (see "Materials and Met experiments. growth was obtained by comparing the growth of cells treated with oligodeoxynucleotide

^a AS, antisense.

 R_{1a} AS (5 μ w) + Random (5 μ w)
 R_{1a} AS (5 μ w) + Random (5 μ w) 1.0 1.0 92 of
 α AS, antisense.

Sequester the protein kinase in an inactive holoenzyme. Thus, the ratio is

19. Secquester the protein **Random (10** μ **M)** 1.0 1.0 92

^{of} AS, antisense.

sequester the protein kinase in an inactive holoenzyme. Thus, the ratio

of type I/type II protein kinase would be regulated mainly by the

amounts of RI_α and RII_β ^a AS, antisense.

sequester the protein kinase in an inactive holoenzyme. Thus, the ratio

of type *II* protein kinase would be regulated mainly by the

amounts of RI_α and RII_β available, when RII_α remains constan sequester the protein kinase in an inactive holoenzyme. Thus, the ratio colons
of type II protein kinase would be regulated mainly by the
amounts of RI_{α} and RII_B available, when RII_α remains constant. That
the do sequester the protein kinase in an inactive holoenzyme. Thus, the ratio
of type I/type II protein kinase would be regulated mainly by the
amounts of RI_{α} and RI_{β} available, when RI_{α} remains constant. That
the d of type I/type II protein kinase in an inactive holocharyme. Thus, the ratio
of type I/type II protein kinase would be regulated mainly by the
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 amounts of RI_{α} and RII_{β} available, when RII_{α} remains constant. That 20. The down-regulation of RI_{α} led to growth inhibition, as shown by RI_{α} remainsee oligodeoxynucleotide or by 8-Cl-cAM the down-regulation of RI_{α} led to growth inhibition, as shown by RI $_{\alpha}$
antisense oligodeoxynucleotide or by 8-CI-cAMP treatment, suggests
that RI_{α} is a positive regulator of cell growth. Down-regulation of
bo antisense oligodeoxynucleotide or by 8-CI-cAMP treatment, suggests
that RI_{α} is a positive regulator of cell growth. Down-regulation of
both RI_{α} and RI_{β} or RI_{β} alone leads to continued cell growth, while
 RI and RI_α is a positive regulator of cell growth. Down-regulation of
both RI_{α} and RII_{β} or RII_{β} alone leads to continued cell growth, while
 RII_{β} overexpression results in growth inhibition (33), suggesting both RI_{β} and RI_{β} or RI_{β} alone leads to continued cell growth, while
 RI_{β} overexpression results in growth inhibition (33), suggesting that
 RI_{β} is a negative regulator of cell growth. The RI_{α} antis RII_β overexpression results in growth inhibition (33), suggesting that

REFERENCES

-
- ancer cells.
 I. Krebs, E. G. Protein kinase. Curr. Top. Cell Regul., 5: 99–133, 1972.

2. Krebs, E. G., and Beavo, J. A. Phosphorylation-dephosphorylation of enzymes. Annu.

Rev. Biochem., 48: 923–939, 1979. REFERENCES

1. Krebs, E. G. Protein kinase. Curr. Top. Cell Regul., 5: 99–133, 1972.

2. Krebs, E. G., and Beavo, J. A. Phosphorylation-dephosphorylation of enzymes. Annu.

Rev. Biochem., 48: 923–939, 1979.

3. Beebe, S. J
- 2. Krebs, E. G. Protein kinase. Curr. Top. Cell Regul., 5: 99–133, 1972.

2. Krebs, E. G., and Beavo, J. A. Phosphorylation-dephosphorylation of enzymes. Annu.

2. Krebs, E. G., and Enzymes. A. Phosphorylation, dephosphory Krebs, E. G. Protein kinase. Curr. Top. Cell Regul., 5: 99–
Krebs, E. G., and Beavo, J. A. Phosphorylation-dephosphory
Rev. Biochem., 48: 923–939, 1979.
Beebe, S. J., and Corbin, J. D. Cyclic nucleotide-dependent
Boyer and
- 25. Kress, E. G., and Beavo, J. A. Prosphorylation-dephosphorylation of enzymes. Annu.

Rev. Biochem., 48: 923–939, 1979.

3. Beebe, S. J., and Corbin, J. D. Cyclic nucleotide-dependent protein kinases. In: P. D.

Boyer an
- Soler and E. O. Keroes (eds.), the Enzymies: Control by Phosphorylation, Part A,

Vol. 17, pp. 43-111. New York: Academic Press, 1986.

4. McKnight, G. S., Clegg, C. H., Uhler, M. D., Chrivia, J. C., Cadd, G. G., Correll, molecular genetic approaches. Recent Prog. Horm
Levy, F. O., Øyen, O., Sandberg, M., Taskén, Jahnsen, T. Molecular cloning, complementary de
predicted full-length amino acid sequence of the lunit of 3.'-5'-cyclic adenosine 6. Levy, F. O., Wyen, O., Sandoerg, M., 188ken, N., Eskild, W., Hansson, V., and Jahnsen, T. Molecular cloning, complementary desvytibonucleic acid structure and predicted full-length amino acid sequence of the hormone-ind
- predicted full-length amino acid sequence of the hormone-inducible regulatory sub-
unit of 3,'-5'-cyclic adenosine monophosphate-dependent protein kinase from human
testis. Mol. Endocrinol., 2: 1364-1373, 1988.
Øyen, O., S unit of 3,'-5'-cyclic adenosine monophosphate-dependent protein kinase from human
testis. Mol. Endocrinol., 2: 1364–1373, 1988.
6. Øyen, O., Scott, J. D., Cadd, G. G., McKnight, G. S., Krebs, E. G., Hansson, V., and
ahnsen testis. Mol. Endocrinol., 2: 1364–1373, 1988.

Øyen, O., Scott, J. D., Cadd, G. G., McKnight, G. S., Kret

Jahnsen, T. A unique mRNA species for a regulatory sul

protein kinase is specifically induced in haploid germ cel
 7. Guyen, O., Scott, J. D., Cadd, G. G., McKnight, G. S., Krebs, E. G., Hansson, V., and Jahnsen, T. A unique mRNA species for a regulatory subunit of cAMP-dependent protein kinase is specifically induced in haploid germ c Jahnsen, T. A unique mRNA species for a regulatory subunit of cAMP-dependent
protein kinase is specifically induced in haploid germ cells. FEBS Lett., 229: 391–
394, 1988.
Cadd, G., and McKnight, G. S. Distinct patterns of protein kinase is specifically induced in haploid germ cells. FEBS Lett., 229: 391-
-
- protein kinase is specifically induced in haploid germ cells. FBBS Lett., 229: 391–30.
Cadd, G., and McKnight, G. S. Distinct patterns of cAMP-dependent protein kinase
gene expression in mouse brain. Neuron, 3: 71–79, 1989 1990. gene expression in mouse brain. Neuron, 3: 71–79, 1989.

8. Cadd, G. G., Uhler, M. D., and McKnight G. S. Holoenzymes of cAMP-depend-

ent protein kinase containing the neural form of type I regulatory subunit have

an inc Cadd, G. G., Uhler, M. D., and McKnight G. S. Holoenzymes of cAMP-dependent protein kinase containing the neural form of type I regulatory subunit have an increased sensitivity to cyclic nucleotides. J. Biol. Chem., 265: 1
- ent protein kinase containing the neural form of type
an increased sensitivity to cyclic nucleotides. J. Biol. (
1990.
Kapoor, C. L., and Cho-Chung, Y. S. Compartmentalizatic
syclic adenosine 3':5'-monophosphate-dependent an increased sensitivity to cyclic nucleotides. J. Biol. Chem., 205: 1950Z-1950
1990.
9. Kapoor, C. L., and Cho-Chung, Y. S. Compartmentalization of regulatory subunits cyclic adenosine 3':5'-monophosphate-dependent protei
- 1990.
Kapoor, C. L., and Cho-Chung, Y. S. Compartmentalization of regulatory subunits of
syclic adenosine 3':5'-monophosphate-dependent protein kinases in MCF-7 human
breast cancer cells. Cancer Res., 43: 295-302, 1983.
Ka cyclic adenosine 3':5'-monophosphate-dependent protein kinases in MCF-7 human
breast cancer cells. Cancer Res., 43: 295-302, 1983.
10. Kapoor, C. L., and Cho-Chung, Y. S. Mitotic apparatus and nucleoli compartmental-
izati

- 11. Nigg, E. A., Schäfer, G., Hilz, H., and Eppenberger, H. M. Cyclic-AMP-dependent

11. Nigg, E. A., Schäfer, G., Hilz, H., and Eppenberger, H. M. Cyclic-AMP-dependent

protein kinase type II is associated with the Golgi Rep., 7: 49–60, 1983.

Rep., 7: 49–60, 1983.

Nigg, E. A., Schäfer, G., Hilz, H., and Eppenberger, H. M. Cyclic-AMP-dependent

protein kinase type II is associated with the Golgi complex and with centrosomes.

Cell, 41: 10
- Rep., 7: 49-60, 1983.

11. Nigg, E. A., Schäfer, G., Hilz, H., and Eppenberger, H. M. Cyclic-AMP-dependent

protein kinase type II is associated with the Golgi complex and with centrosomes.

Cell, 41: 1039-1051, 1985.

12. protein kinase type II is associated with the Golgi complex and with centrosomes.
Cell, 41: 1039–1051, 1985.
Lohmann, S. M., and Walter, U. Regulation of the cellular and subcellular concentrations and distribution of cycl potent kinase type in is associated with the Gorg complex an
Cell, 41: 1039–1051, 1985.
Lohmann, S. M., and Walter, U. Regulation of the cellular and
trations and distribution of cyclic nucleotide-dependent protein la
gard 12. Lohmann, S. M., and Walter, U. Regulation of the cellular and subcellular concentrations and distribution of cyclic nucleotide-dependent protein kinases. In: P. Greenard et al. (eds.), Advances in Cyclic Nucleotide an Examing of the interiors and distribution of cyclic nucleotide-dependent protein kinases. In: P. Greengard et al. (eds.), Advances in Cyclic Nucleotide and Protein Phosphorylation Research, Vol. 18, pp. 63-117. New York: R
- 14. Roleh, N., Johnson, G., Laughlin, C., Green. I. The Spinorylator Research, Nol. 18, pp. 63-117. New York: Raven Press, 1984.

13. Cho-Chung, Y. S. Perspectives in cancer research: role of cyclic AMP receptor proteins i
- expression: therapeutic implication, and suppression of malignancy: new approaches in growth, differentiation, and suppression of malignancy: new approaches to therapy. Cancer Res., 50: 7093-7100, 1990.
Rothenberg, M., Joh process in grown, direcentiation, and suppression of mangnancy: new approaches to
therapy. Cancer Res., 50: 7093-7100, 1990.
Rothenberg, M., Johnson, G., Laughlin, C., Green, I., Craddock, J., Sarver, N., and
Cohen, J. S.
- merapy. Cancer Res., 30. 1093–1100, 1990.
Rothenberg, M., Johnson, G., Laughlin, C., Green, I., Craddock, J., Sarver, N., and
Cohen, J. S. Commentary: oligodeoxynucleotides as anti-sense inhibitors of gene
expression: ther Kolnenberg, M., Johnson, G., Laugnin, C., Green, I., Craddock, J., Sarver, N., and
Cohen, J. S. Commentary: oligodeoxynucleotides as anti-sense inhibitors of gene
expression: therapeutic implications. J. Natl. Cancer Inst. 1991.
16. Tortora, G., Clair, T., and Cho-Chung, Y. S. An antisense oligodeoxynucleotide Expression: therapeutic implications. J. Nati. Cancer Inst., 61: 1339–1344, 1989.

15. Tortora, G., Yokozaki, H., Pepe, S., Clair, T., and Cho-Chung, Y. S. Differentiation

of HL-60 leukemia by type I regulatory subunit a
- For the type Rigulatory subunit antisense oligodeoxynucleotide of cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA, 88: 2011-2015, 1991.
Tortora, G., Clair, T., and Cho-Chung, Y. S. An antisense oligodeoxynucleoti or exter-dependent protein kinase. Proc. Nati. Acad. Sci. USA, 1991.
Tortora, G., Clair, T., and Cho-Chung, Y. S. An antisense olig
targeted against the type RII_B regulatory subunit mRNA of prote
cAMP-induced differentia 17. Radhamahakarishnan. P. I. and Cho-Chung, Y. S. An antisense oligodeoxynucleotide targeted against the type RII_B regulatory subunit mRNA of protein kinase inhibits cAMP-induced differentiation in HL-60 leukemia cells
- iargeted against the type Kii_B regulatory subunit mich/A of protein kinase innibits
CAMP-induced differentiation in HL-60 leukemia cells without affecting phorbol
ester effects. Proc. Natl. Acad. Sci. USA, 87: 705-708, 1 1990. 18. Radhakrishnan, P. I., Egan, W., Regan, J. B., and Beaucage, S. L. 3H-1,2-benzodithi-
18. Radhakrishnan, P. I., Egan, W., Regan, J. B., and Beaucage, S. L. 3H-1,2-benzodithi-
1990.
18. Lowry, O. H., Rosebrough, N. J., F Radinakrismian, F. 1., Egan, W., Regan, J. B., and Beaucage, S. L. 3*H*-1,2
ole-3-one 1,1-dioxide as an improved sulfurizing reagent in the solid-pha
of oligodeoxyribonucleoside phosphorothioates. J. Am. Chem., Soc., *112* 19. Sandberg. M.. Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement

19. Sandberg. M., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement

19. Sandberg, M., Taskén, K., Øyen, O., Ha
-
- consumed and deduced amino acid sequence for a type I regulatory of H. P. Structure for a type I regulatory subunit of cAMP-dependent protein kinase from human testis. Biochem. Biophys. Res. Computer can the cAMP-dependent with the Folin phenol reagent. J. Biol. Chem., 193: 265–275, 1951.
Sandberg, M., Taskén, K., Øyen, O., Hansson, V., and Jahnsen, T. Molecular cloning, cDNA structure and deduced amino acid sequence for a type I regulatory Sandberg, M., 18sken, K., *yyen*, O., riansson, V., and Jamisen, 1. Molecular cloning
CDNA structure and deduced amino acid sequence for a type I regulatory subunit o
CAMP-dependent protein kinase from human testis. Bioche CDNA Structure and deduced amino acid sequence for a type 1 regulatory subunit
 $cAMP-dependent protein kinase from human testis. Biochem. Biophys. Res. Cor
\nmun., 149: 939–945, 1987.
\n20. Maldonado, F., and Hanks, S. K. A cDNA clone encoding human cAMP-depend
\nprotein kinase catalytic subunit $C\alpha$. Nucleic Acids Res., 16: 8189–8190, 1988.
\n21. Thuong, N. T., Assel$
- 20. Maldonado. F., and Hanks. S. K. A cDNA clone encoding human cAMP-dependent
- CAMP-dependent protein kinase from numan testis. Biochem. Biophys. Kes. Com-
mun., 149: 939–945, 1987.
Maldonado, F., and Hanks, S. K. A cDNA clone encoding human cAMP-dependent
protein kinase catalytic subunit Cα. Nuclei munt, 1757, 2007–2007. 1907. ISON Achone encoding human cAMP-dependent protein kinase catalytic subunit C α . Nucleic Acids Res., 16: 8189–8190, 1988. Thuong, N. T., Asseline, U., Roig, V., Takasugi, M., and Hélène, C. Ol protein kinase catalytic subtint Ca. Nucleic Acids Res., 10: 8189-8150, 17868.
21. Thuong, N. T., Asseline, U., Rojg, V., Takasugi, M., and Hélène, C. Oligo(α -deoxynucleotide) scovalently linked to intercalating agents: nucleotide)s covalently linked to intercalating agents: differential binding to ribo- and deoxyribopolynucleotides and stability towards nuclease digestion. Proc. Natl. Acad. Sci. USA, 84 : 5129-5133, 1987.
Campbell, J. M
- J. Biochem. Biophys. Methods, 20: 259-267, 1990. 22. Campbell, J. M., Bacon, T. A., and Wickstrom, E. Oligodeoxynucleoside phospho-Sci. USA, 84: 5129–5133, 1987.
Campbell, J. M., Bacon, T. A., and Wickstrom, E. Oligodeoxynucleoside phosphorothioate stability in subcellular extracts, culture media, sera, and cerebrospinal fluid.
J. Biochem. Biophys. Me
- Campoen, J. M., Bacon, 1. A., and Wickstrom, E. Ongodeoxynucleoside phosphotochioate stability in subcellular extracts, culture media, sera, and cerebrospinal fluid.
J. Biochem. Biophys. Methods, 20: 259–267, 1990.
Nowak, 1987. 23. Nowak, I., Seipel, K., Schwarz, M., Jans, D. A., and Hemmings, B. A. Isolation of a cDNA and characterization of the 5' flanking region of the gene encoding the type I regulatory subunit of the cAMP-dependent protein k
- regulatory subunit of the cAMP-dependent protein kinase. FEBS Lett., 167: 27–33, 1987.
Ally, S., Tortora, G., Clair, T., Grieco, D., Merlo, G., Katsaros, D., Ogreid, D., Doskeland, S. O., Jahnsen, T., and Cho-Chung, Y. S. plays.
1987.
1987. Control of the Crivit-Seperincin protein Kinase. 1 EBS Ect., 107. 21–53,
Ally, S., Tortora, G., Clair, T., and Cho-Chung, Y. S. Selective modulation of protein
kinase isozymes by the site-selective analo 24. Ally, S., Iortora, G., Clair, 1., Grieco, D., Merlo, G., Katsaros, D., Ogreid, D., Doskeland, S. O., Jahnsen, T., and Cho-Chung, Y. S. Selective modulation of protein kinase isozymes by the site-selective analog 8-chlo Doskeland, S. O., Jahnsen, T., and Cho-Chung, Y. S. Selective modulation of protein
kinase isozymes by the site-selective analog 8-chloroadenosine 3'.5'-cyclic mono-
phosphate provides a biological means for control of hum
-
- phosphate provides a biological means for control of human colon cancer cell growth.
Proc. Natl. Acad. Sci. USA, 85: 6319–6322, 1988.
Hofmann, F., Bechel, P. J., and Krebs, E. G. Concentrations of cyclic AMP-dependent
prot Froc. Ivan. Acad. Scl. OSA, 80: 0319-0322, 1968.
Hofmann, F., Bechtel, P. J., and Krebs, E. G. Concentrations of cyclic AMP-dependent
protein kinase subunits in various tissues. J. Biol. Chem., 252: 1441-1447, 1977.
Cho-Ch Holmann, F., Bechtel, P. J., and Krebs, E. G. Concentrations of cyclic Ahrodein kinase subunits in various tissues. J. Biol. Chem., 252: 1441–Cho-Chung, Y. S., Clair, T., Tagliaerri, P., Ally, S., Katsaros, D., Totrotae
L. protein kinase subunits in various tissues. J. Biol. Chem., 252: 1441-1447, 1977.
26. Cho-Chung, Y. S., Clair, T., Tagliaferri, P., Ally, S., Katsaros, D., Tortora, G., Neckers,
L., Avery, T. L., Crabtree, G. W., and Robin Cho-Chung, Y. S., Clair, I., Iaguaterri, P., Aily, S., Katsaros, D., 16
L., Avery, T. L., Crabtree, G. W., and Robins, R. K. Basic sc
selective cyclic AMP analogs as new biological tools in growth con-
and proto-oncogene r 28. Weber, W., E., Chauchee, G., with Nobilas, K. K. Basic Scheine Fevrew. sue-
selective cyclic AMP analogs as new biological tools in growth control, differentiation
and proto-oncogene regulation. Cancer Invest., 7: 161–
-
- proto-oncogene regulation. Cancer Investigations in growom contomol, directed
and proto-oncogene regulation. Cancer Investigated Friedman, D. L., and Strittholt, J. T. Adenosine 3':5'-monophosphate receptor protein
in HeLa and proto-oncogene regulation. Cancer Invest., 7: 161–177, 1989.

27. Friedman, D. L., and Strittholt, J. T. Adenosine 3':5'-monophosphate receptor protein

in HeLa cells. Biochim. Biophys. Acta, 675: 334–343, 1981.

28. W 29. Weber, W., Schwoch, G., and Hilz, H. Aotensine 3.34–343, 1981.

28. Weber, W., Schwoch, G., and Hilz, H. Isolation of a 50,000 dalton cAMP binding

protein and its characterization as regulatory subunit of protein kin
- Weber, W., Schwoch, G., and Hilz, H. Isolation of a 50,000 daiton cAprocein and its characterization as regulatory submit of protein kinase Biophys. Res. Commun., 104: 1134–1141, 1982.
Weber, W., Schwoch, G., Wielekers, K.
- Biophys. Res. Commun... 104: 1134-1141, 1982.

29. Weber, W., Schwoch, G., Wielekers, K., Gartemann, A., and Hilz, H. c.AMP receptor

29. Weber, W., Schwoch, G., Wielekers, K., Gartemann, A., and Hilz, H. c.AMP receptor

p 1. provision and protein kinases in numan typinocytes: tuneamental atteration in circinculation, purification, and characterization of subunits of cAMP-dependent protein kinase in human testis. J. Biol. Chem., 267: 5374–53
- Jahnsen, T. Identification, purification, and characterization of subunits of cAMP-
dependent protein kinase in human testis. J. Biol. Chem., 267: 5374–5379, 1992.
Otten, A. D., and McKnight, G. S. Overexpression of the t of the cAMP-dependent protein kinase eliminates the type I holoenzyme in mouse
- 32. Otten, A. D., Parenteau, L. A., Doskeland, S., and McKnight, G. S. Hormonal activation of gene transcription in ras-transformed NIH3T3 cells overexpressing RII_{α} and RII_{β} subunits of the cAMP-dependent protein Otten, A. D., and McKnight, G. S. Overexpression of the type II regulatory subunit
of the cAMP-dependent protein kinase eliminates the type I holoenzyme in mouse
cells. J. Biol. Chem., 264: 20255-20260, 1989.
Otten, A. D. of the cAMP-dependent protein kinase eliminates the type 1 holoenzyme in mouse

22. Otten, A. D., Parenteau, L. A., Doskeland, S., and McKnight, G. S. Hormona

23. Otten, A. D., Parenteau, L. A., Doskeland, S., and McKnig Otten, A. D., Parenteau, L. A., Doskeland, S., and McKnight, G. S. Hormona
activation of gene transcription in ras-transformed NIH3T3 cells overexpressing RII,
and RII_p subunits of the cAMP-dependent protein kinase. J.
- activation of gene transcription in ras-transformed NIH313 cells overexpressing KII_a and RII_B subunits of the cAMP-dependent protein kinase. J. Biol. Chem., 266:
23074–23082, 1991.
Rohlff, C., Clair, T., and Cho-Chung, and KII_B subunits of the cAMP-dependent protein kinase. J. Biol. Chem., .
23074–23082, 1991.
Rohlff, C., Clair, T., and Cho-Chung, Y. S. 8-CI-cAMP induces truncation and do
regulation of the RI_a subunit and up-regulati

Cancer Research The Journal of Cancer Research (1916-1930) | The American Journal of Cancer (1931-1940)

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Hiroshi Yokozaki, Alfredo Budillon, Giampaolo Tortora, et al.

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