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13. For sequencing, the inserts were subcloned into the Bluescript KS (+) plasmid vector (Stratagene). Overlapping deletions were generated with Exo III and S1 nucleases (double-stranded nested deletion kit, Pharmacia) and sequenced on both strands with the dideoxynucleotide procedure of F. Sanger, S. Nicklen, and A. R. Coulson [*Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)]. Compressed DNA regions were sequenced using the fmol DNA sequencing system (Promega). DNA sequence analysis was done on a VAX with the GCG program [J. Devereux, P. Haeberli, O. Smithies, *Nucleic Acids Res.* **12**, 387 (1984)]. The homology searches through the EMBL and SWISSPROT data banks were carried out with the FASTA programs [W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2444 (1988)]. EMBL Data Library accession numbers: X79492 *D. melanogaster ey* mRNA (exon 1) and X79493 *D. melanogaster ey* mRNA (exons 2–9).
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26. This result was confirmed by in situ hybridization of sense and antisense RNA probes to wild-type, ey^2, and ey^R larvae. In the majority of the mutant eye disks, no hybridization of antisense RNA was detected above the background that was found with sense RNA. In some of the mutant disks, a few scattered positive cells were detected, which is in agreement with the variable expression of the mutant phenotype (R. Leemans and W. Gehring, unpublished data).
27. The 3.6-kb Eco RI restriction fragment flanking the insertion sites was cloned into HZ50PL [Y. Hiromi and W. J. Gehring, *Cell* **50**, 963 (1987)], and transgenic lines were established (U. Walldorf and W. J. Gehring, unpublished data).
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31. A gene with significant homology to *Pax-6* in both the paired box and the homeobox was cloned from a genomic DNA library of the nemertean worm *Lineus sanguineus* (F. Loosli, M. T. Kmita, W. J. Gehring, unpublished data).
32. Sequences homologous to the paired box of *Pax-6* were PCR-amplified, cloned, and sequenced from DNA isolated from squid (*L. vulgaris*) sperm (P. Callaerts, J. Marthy, W. J. Gehring, unpublished data).
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Blockage of NF-κB Signaling by Selective Ablation of an mRNA Target by 2-5A Antisense Chimeras

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Activation of 2-5A–dependent ribonuclease by 5′-phosphorylated, 2′,5′-linked oligoadenylates, known as 2-5A, is one pathway of interferon action. Unaided uptake into HeLa cells of 2-5A linked to an antisense oligonucleotide resulted in the selective ablation of messenger RNA for the double-stranded RNA (dsRNA)–dependent protein kinase PKR. Similarly, purified, recombinant human 2-5A–dependent ribonuclease was induced to selectively cleave PKR messenger RNA. Cells depleted of PKR activity were unresponsive to activation of nuclear factor–κB (NF-κB) by the dsRNA poly(I):poly(C), which provides direct evidence that PKR is a transducer for the dsRNA signaling of NF-κB.

Natural defense mechanisms can be allies in the quest for therapeutic approaches to disease. One such defense, the 2-5A system (*1*), mediates certain effects of interferons, such as the inhibition of encephalomyocarditis virus replication (*2*). Key components of this system include 2-5A, short oligoadenylates with 2′,5′-phosphodiester bonds; 2-5A synthetases that generate 2-5A from adenosine triphosphate (ATP) in response to dsRNA; and the effector of the system, the 2-5A–dependent ribonuclease (RNase) (*3*, *4*). This RNase, which is ubiquitous in the cells of mammalian, reptilian, and avian species (*5*), cleaves single-stranded RNA in response to 2-5A, with moderate specificity after UpNp sequences (*6*). Thus, this host defense mechanism is the basis for a strategy for the selective destruction of specific mRNA targets.

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We demonstrate here that a natural mRNA that encodes the dsRNA-dependent protein kinase PKR (*7*) can be selectively destroyed when human cells are treated with micromolar concentrations of 2-5A linked to antisense oligonucleotides (*8*). PKR is an interferon-induced, serine-threonine protein kinase that is stimulated by dsRNA to phosphorylate protein synthesis initiation factor eIF2α (*7*). Phosphorylation of eIF2α blocks an eIF2B-catalyzed recycling reaction resulting in an inhibition in translation (*9*). Like the 2-5A system, PKR is implicated in the anti-encephalomyocarditis virus effect of interferon (*10*). Because the expression of mutant forms of PKR in NIH 3T3 cells produces tumors in nude mice, PKR may also function as a tumor suppressor (*11*). Furthermore, studies with the protein kinase inhibitor 2-aminopurine have suggested that PKR is involved in the dsRNA-mediated activation of the interferon β gene and in the viral induction of interferon genes (*12*). The dsRNA treatment of cells activates transcription factor NF-κB (*13*), probably as a result of the phosphorylation of its inhibitory protein, IκBα, by PKR (*14*). To obtain direct evidence for the involvement of PKR in dsRNA-mediated signal transduction, we selected PKR mRNA as a target for the 2-5A antisense strategy.

To determine the activity of 2-5A antisense against PKR mRNA in intact human cells, we selected a binding site for the chimeras that was 55 to 73 nucleotides from the start codon of the PKR mRNA. The chimera, 5′-p(A2′p)$_3$A-linker-GTACTACT-CCCTGCTTCTG-3′, called p(A2′p)$_3$A-antiPKR, was synthesized as described (*8*, *15*, *16*). The linker consisted of two 1,4-butanediol monomers attached to each other and to the p5′(A2′p5′)$_3$A and 3′,5′-oligodeoxyribonucleotide moieties with phosphodiester bonds (*8*, *15*). All of the chimeras described in this study were constructed in a similar fashion (*16*). We designed two chimeras to stabilize the 5′-phosphoryl group and antisense moieties to phosphatase or phosphodiesterase activities, respectively. In one analog, the 5′-thiophosphate, Sp(A2′p)$_3$A-antiPKR, the O double-bonded to the P in the 5′-phosphoryl group was replaced with S. In the other chimera, p(A2′p)$_3$A-antiPKR–3′-tail, the 3′-terminus was protected from 3′- to 5′-exonucleases by the addition of a 3′-tailing group. This 3′-tailing group was added by linking the 3′-hydroxyl group of the 3′-terminal antisense nucleotide through a phosphodiester bond to the primary hydroxyl moiety of 1-amino-2,3-propanediol.

PKR mRNA decay was induced by the addition of the oligonucleotides (at 2 μM) to cultures of HeLa cells without any facilitation of uptake. PKR mRNA was detected by the reverse transcriptase–coupled polymerase chain reaction (RT-PCR) (*17*). No PKR mRNA was detected after incubation of the cells for 4 hours with Sp(A2′p)$_3$A-antiPKR, p(A2′p)$_3$A-antiPKR–3′-tail, or p(A2′p)$_3$A-antiPKR (Fig. 1A, left panel). In contrast, the control oligonucleotides, antiPKR (the antisense moiety by itself), p(A2′p)$_3$A-antiHIV [containing an antisense to human immunodeficiency virus (HIV) RNA that was unrelated to the PKR sequence], or p(A2′p)$_3$A-(dA)$_{18}$ [directed to oligo(rU)] had no effect on PKR mRNA amounts (Fig. 1A, left panel). The amounts of PKR RNA in cells exposed to one dose of 2 μM Sp(A2′p)$_3$A-antiPKR, p(A2′p)$_3$A-antiPKR–3′-tail, or p(A2′p)$_3$A-antiPKR returned to normal by 36 hours. Therefore, under these conditions, the modifications of the chimeras did not appear to significantly prolong the depletion of PKR mRNA. To determine if the 2-5A antisense was selective for PKR mRNA, we also measured the amounts of β-actin mRNA (*18*) by the same method (Fig. 1A, right panel). None of the oligonucleotides tested depleted the amount of β-actin mRNA.

The ablation of PKR mRNA was confirmed in RNase protection assays performed with RNA isolated from HeLa cells treated for 4 hours with 2 μM p(A2′p)$_3$A-antiPKR or p(A2′p)$_3$A-sensePKR (containing sense orientation oligonucleotides for PKR mRNA, from nucleotides 55 to 73) (*19*). PKR mRNA amounts were depleted after treatment of cells with p(A2′p)$_3$A-antiPKR, whereas p(A2′p)$_3$A-sensePKR was without effect (Fig. 1B). In addition, neither 2 μM (A2′p)$_3$A-antiPKR, which lacks the 5′-phosphoryl group necessary for efficient activation of the human form of 2-5A–dependent RNase (*20*), nor the unmodified antisense to the PKR alone produced a significant depletion of PKR mRNA under the same conditions (*21*).

We performed kinetic experiments to measure the decline and subsequent recovery of PKR mRNA amounts after treatment of the cells with a single dose of 2 μM p(A2′p)$_3$A-antiPKR (Fig. 1C). About 4 hours of treatment were required to reduce PKR mRNA amounts to minimal levels, (to

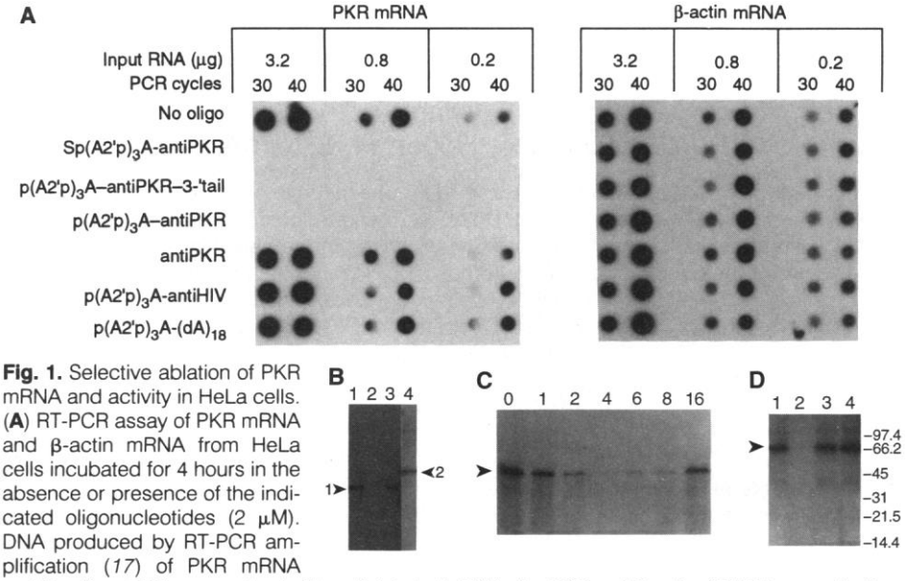

Fig. 1. Selective ablation of PKR mRNA and activity in HeLa cells. (**A**) RT-PCR assay of PKR mRNA and β-actin mRNA from HeLa cells incubated for 4 hours in the absence or presence of the indicated oligonucleotides (2 μM). DNA produced by RT-PCR amplification (*17*) of PKR mRNA and β-actin mRNA was probed with radiolabeled cDNAs for PKR and β-actin. (**B**) RNase protection assay (*19*) for PKR mRNA from HeLa cells incubated for 4 hours in the absence of oligonucleotide (lane 1) or with 2 μM p(A2′p)$_3$A-antiPKR (lane 2) or p(A2′p)$_3$A-sensePKR (lane 3). The free probe is shown in lane 4. The PKR mRNA is indicated by arrow 1 and the probe by arrow 2. (**C**) RNase protection assay for PKR mRNA as a function of hours after treatment (as indicated at the top of each lane) of HeLa cells with 2 μM p(A2′p)$_3$A-antiPKR. Arrow indicates PKR mRNA. Bands were scanned by a PhosphorImager (Molecular Dynamics, Sunnyvale, California). (**D**) Autophosphorylation assay of PKR (*23*) from extracts of HeLa cells incubated for 3.5 days in the absence of oligonucleotide (lane 1) or in the presence of p(A2′p)$_3$A-antiPKR (lane 2), p(A2′p)$_3$A-sensePKR (lane 3), or (A2′p)$_3$A-antiPKR (lane 4). The arrow at left indicates the position of PKR, and the molecular size standards are indicated at right in kilodaltons.

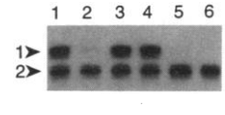

Fig. 2. The 2-5A antisense directs homogenous, recombinant human 2-5A–dependent RNase to selectively cleave the mRNA target (*25*). The positions of the PKR mRNA (2.0 kb) and 2-5A–synthetase mRNA (1.8 kb) are indicated by arrows 1 and 2, respectively. Lane 1, absence of oligonucleotide; lane 2, p(A2′p)$_3$A-antiPKR; lane 3, p(A2′p)$_3$A-sensePKR; lane 4, (A2′p)$_3$A-antiPKR; lane 5, Sp(A2′p)$_3$A-antiPKR; and lane 6, p(A2′p)$_3$A-antiPKR–3′-tail.

less than 2% of the amount before treatment). Subsequently, PKR mRNA amounts began to recover, such that at 16 hours after treatment, there was 56% of the amount of PKR mRNA before treatment. The recovery of PKR mRNA amounts presumably reflects the decay of $p(A2'p)_3A$-antiPKR and the synthesis of PKR mRNA by the cells. Reduction of PKR mRNA amounts in HeLa cells to minimal levels by 4 hours required 2 μM or higher concentrations of $p(A2'p)_3A$-antiPKR (*21*). The RNase protection assays confirmed data obtained with RT-PCR that established that the induction of PKR mRNA decay required the presence in the chimeras of both functional 2-5A and the antisense sequence. Furthermore, in 8 of 13 separate cell cultures, the depletion of PKR mRNA in response to 2-5A antisense appeared complete by 4 hours. In the other five cell cultures, amounts of PKR mRNA were substantially reduced (by >80%) after 4 hours of treatment with 2 μM $p(A2'p)_3A$-antiPKR. Selectivity of 2-5A antisense was established by the finding that $p(A2'p)_3A$-sensePKR did not result in the degradation of PKR mRNA (Fig. 1B). In addition, the treatment of cells with 2 μM $p(A2'p)_3A$-antiPKR did not reduce the amounts of untargeted mRNA species for γ-actin and tyrosine protein kinase JAK1 (*22*), as determined by RNase protection assays (*21*). These chimeras, therefore, do not induce a measurable, nonspecific degradation of mRNA. As a further indication of the selectivity of 2-5A antisense, there was no inhibition of cell proliferation by treatment of the cells with $p(A2'p)_3A$-antiPKR or $p(A2'p)_3A$-$(dA)_{18}$, each used at a single dose of 2 μM, and then cultured for 36 hours (*21*).

Cellular amounts of PKR activity were determined by functional assays after twice daily treatments with various chimeras for 3.5 days (Fig. 1D). PKR was immobilized from the cell extracts and autophosphorylated with [γ-^{32}P]ATP on the activating affinity matrix, poly(I):poly(C)-cellulose (*23, 24*). Treatment of cells with $p(A2'p)_3A$-antiPKR reduced PKR activity to undetectable amounts (Fig. 1D). In contrast, the control chimeras, $p(A2'p)_3A$-sensePKR and $(A2'p)_3A$-antiPKR, did not affect PKR activity.

In order to establish that 2-5A antisense can activate 2-5A–dependent RNase and cause the selective degradation of RNA targets, assays were performed with homogenous, recombinant human 2-5A–dependent RNase expressed from a baculovirus vector in insect cells (*25, 26*). PKR mRNA was mixed with an untargeted mRNA for a small form of human 2-5A–synthetase. Only the PKR mRNA was significantly degraded in response to 25 nM $p(A2'p)_3A$-antiPKR, $Sp(A2'p)_3A$-antiPKR, and $p(A2'p)_3A$-antiPKR–3′-tail (Fig. 2). In contrast, the control oligonucleotides, $p(A2'p)_3A$-sensePKR and $(A2'p)_3A$-antiPKR, were without affect against either type of RNA. These results show that 2-5A antisense can direct 2-5A–dependent RNase to cleave mRNA targets in a selective manner. Substantially higher amounts of 2-5A antisense (>300 nM), however, caused nonspecific degradation of RNA (*27*).

To determine if dsRNA signaling was affected by the depletion of PKR activity, we performed electrophoretic mobility shift assays (EMSAs) after the cells were incubated in the presence or absence of different chimeric oligonucleotides (added twice daily) for 3.5 days with the addition of poly(I):poly(C) for the final 12 hours (*28*). The PRDII element of the human interferon β promoter was used as a probe because it binds to NF-κB (*13*). A specific complex was formed with this probe when a nuclear extract of dsRNA-treated cells was used (Fig. 3, lane 2), but not when an extract from control cells was used (lane 1). No PRDII complex was observed when cells were first incubated for 3 days in the presence of $p(A2'p)_3A$-antiPKR before the addition of poly(I):poly(C) (lane 3). In contrast, the control chimeras, $p(A2'p)_3A$-sensePKR and $(A2'p)_3A$-antiPKR, did not block the dsRNA-mediated activation of NF-κB (lanes 4 and 5). The $p(A2'p)_3A$-antiPKR treatment of cells caused no inhibition of the formation of a complex with the interferon-stimulated response element (*29*) we used as a probe (*30*). Therefore, ablation of PKR did not cause a general inhibition of transcription factors. Furthermore, treatment of HeLa cells with the chimeric oligonucleotides did not prevent the activation of NF-κB by tumor necrosis factor–α (TNF-α), a cytokine that is not obviously related to dsRNA signaling (Fig. 3B) (*31*). There was no effect of $p(A2'p)_3A$-antiPKR or $p(A2'p)_3A$-sensePKR treatment of HeLa cells on the TNF-α signaling pathway (lanes 13 to 16). Therefore, the chimeras are not nonspecific inhibitors of NF-κB activation. Competition with unlabeled PRDII and supershift experiments with antibody to p50 subunit of NF-κB verified that the complex observed was the result of NF-κB binding (Fig. 3).

We have reported the synthesis and characterization of chimeric oligonucleotides obtained by linking $p5'(A2'p5')_3A$ to 3′,5′-oligo$(dT)_{18}$ (*8, 15*). After addition of the chimera to crude extracts of human

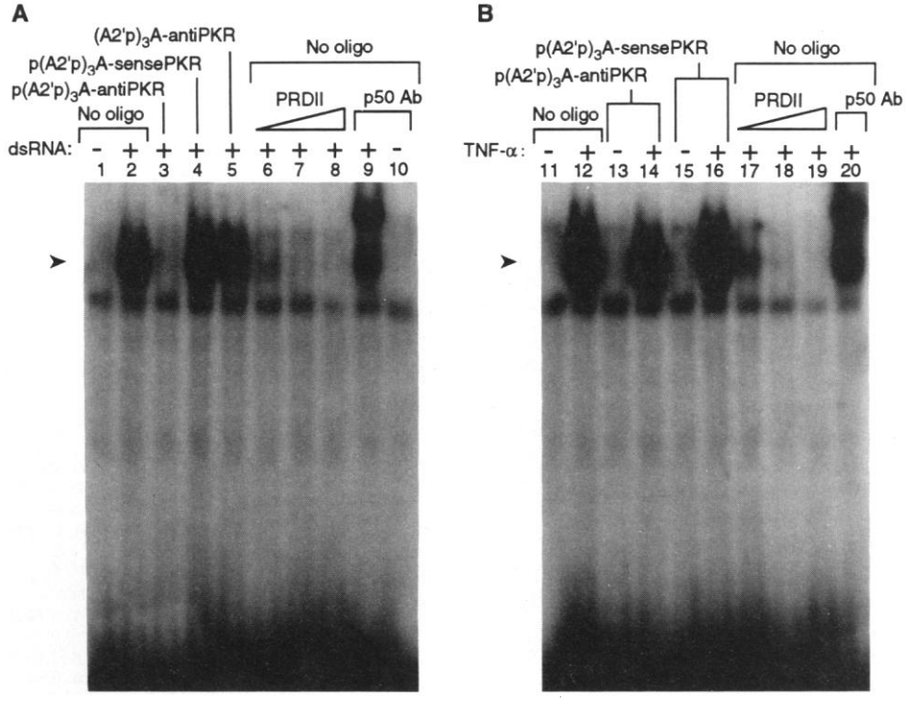

Fig. 3. (**A**) Activation of NF-κB in response to dsRNA is blocked after the depletion of PKR by 2-5A antisense. (**B**) Lack of effect of the chimeric oligonucleotides on NF-κB activation in response to TNF-α. Nuclear extracts from HeLa cells treated (as indicated) were used for EMSAs (*28*). In lanes 6 through 8, 17 through 19, 0.5 ng, 1 ng, and 2.5 ng of cold PRDII DNA oligonucleotide competitor (as compared with 0.1 ng of radiolabeled PRDII probe; ~30,000 cpm) were added. In lanes 9 and 20, we added antibody (Ab) to p50 subunit of NF-κB to nuclear extracts of poly(I):poly(C)-treated cells or TNF-α–treated cells. In lane 10, we added p50 antibody to a nuclear extract of control cells. Arrowheads indicate the migration of the NF-κB complex.

lymphoblastoid Daudi cells, the 2-5A component of the chimera activated 2-5A–dependent RNase, whereas the oligo(dT) portion directed the enzyme to an oligo(rA) sequence inserted into a modified HIV vif mRNA. The result was site-specific degradation of the target RNA molecules (*8*). We have shown here that the 2-5A antisense strategy is active against a naturally occurring mRNA target in intact cells. Ablation of both PKR mRNA and activity was thus obtained. These chimeric oligonucleotides harness the 2-5A–dependent RNase by activating and directing it to an RNA target. Furthermore, we have also demonstrated that 2-5A antisense acts catalytically (*27*). In assays with purified, recombinant 2-5A–dependent RNase, we have shown that the cleavage reactions can be driven to near completion in a 5-min incubation at 37°C under conditions of a 30:1 molar ratio of PKR mRNA to $p(A2'p)_3$A-antiPKR (*27*). These findings demonstrate that 2-5A antisense chimeras can be used as reagents to obtain the catalytic destruction of target mRNA molecules. By specifically blocking the synthesis of disease-causing proteins, 2-5A antisense chimeras may prove to be of therapeutic benefit in a range of diseases.

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17. Cells cultured in Dulbecco's minimum Eagle's medium plus 10% fetal calf serum were incubated in microtiter wells (24-well plates with 250 μl of media per well) in the absence or presence of 2 μM oligonucleotides (as indicated in Fig. 1A) for 4 hours. Total RNA was isolated from the cells with the use of RNazol reagent (Tel-Test, Friendswood, TX). The RNA was then treated with RNase-free deoxyribonuclease (DNase) (Boehringer Mannheim), extracted with phenol-chloroform, and precipitated with ethanol. The mRNA was reverse transcribed into complementary DNA (cDNA) with oligo(dT) used as a primer, and then the cDNA was diluted three times with buffer 10× PCR buffer (Perkin-Elmer, Norwalk, CT) at a final concentration of 1:4. The DNA at each dilution was amplified with the use of primers to antisense and sense sequences at opposite ends of the PKR coding sequence, 5′-CCGTCAGAAGCAGGGAGTAG-3′ and 5′-CCACACAGTCAAGGTCCTTA-3′, respectively, or to the β-actin coding sequence (*18*), 5′-GCTGTGCTATCCCTGTACG-3′ and 5′-TGCCTCAGGGCAGCGGAA-3′, respectively, producing DNA fragments of 1567 base pairs (bp) and 368 bp for PKR cDNA and β-actin cDNA, respectively. The amounts of total RNA we used to produce the cDNAs are indicated (Fig. 1A). To detect the amplified cDNAs, we blotted them to Nytran membranes (Micron Separations, Westboro, MA) and then probed the membranes with PKR cDNA (*7*) or β-actin cDNA (*18*) radiolabeled with α-^{32}P–labeled deoxycytidine 5′-triphosphate (dCTP) by random priming. The anti-PKR sequence is 5′-GTACTACTCCCTGCTTCTG-3′, and the antiHIV sequence is 5′-ACACCCAATTCTGAAATGAA-3′.
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19. HeLa cells were incubated for 4 hours in the absence or presence of 2 μM oligonucleotide (as indicated in Fig. 1B) in 100-mm petri dishes in 5 ml of media. PKR antisense RNA probe was prepared from a Bam HI–digested pGEM-8 plasmid (Promega, Madison, WI) containing PKR cDNA sequence (*7*) from nucleotides −26 to 383 (first nucleotide of the coding sequence is number one) with SP6 RNA polymerase. The RNA probe (3 × 10^5 cpm) was labeled with [α-^{32}P]CTP, and 50 μg of total cellular RNA were used per assay. After hybridization at 45°C for 16 hours, the RNA was digested with RNases A and T1 for 30 min at 30°C, and then treated with proteinase K at 37°C for 15 min. The RNA products were then purified by phenol extraction and were applied to a 6% polyacrylamide–urea gel. The length of the probe was 452 nucleotides, and the protected fragment was 409 nucleotides in length. The sequence for sensePKR is 5′-CAGAAGCAGGGAGTAGTAC-3′.
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23. Chimeric oligonucleotides (2 μM per treatment) were added to cultures of HeLa cells at 0, 12, 24, 36, 48, 60, and 72 hours, and the cells were harvested after 84 hours of incubation. PKR in the cell extracts was immobilized on activating affinity matrix poly(I):poly(C)-cellulose (*24*). Cell extracts (50 μg of protein per assay) were incubated with poly(I):poly(C)-cellulose for 1 hour on ice, after which the matrix was washed three times and incubated in the presence of [γ-^{32}P]ATP (2 μCi per assay; 15 Ci/mmol), 20 mM Hepes (pH 7.5), 80 mM KCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 0.1 mM 2-mercaptoethanol in a final volume of 20 μl for 30 min at 30°C. The proteins were separated on an SDS–10% polyacrylamide gel and analyzed by autoradiography.
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25. The 2-5A–dependent RNase was expressed in SF21 insect cells from a human cDNA (*4*) subcloned in a baculovirus vector (Clontech) and was purified to homogeneity with the use of three fast protein liquid chromatography (FPLC) columns (Pharmacia) (*26*). Cloned human cDNAs for PKR (*7*) and 2-5A–synthetase (a complete coding region cDNA for the human 2-5A–synthetase produced from a 1.8-kb mRNA) in linearized plasmids were transcribed with T7 RNA polymerase (Bethesda Research Laboratories). Each RNA (~200 μg) was dephosphorylated with alkaline phosphatase (Boehringer), incubated with proteinase K, and phenol-extracted before labeling at the 5′-termini with 2 U of T4 polynucleotide kinase (U.S. Biochemicals) and 50 μCi of [γ-^{32}P]ATP (3000 Ci/mmol). The full-length transcripts were purified from 6% polyacrylamide–8 M urea gels for use in the cleavage reactions. Reactions were performed in the absence or presence of chimeric oligonucleotides (25 nM) with both 5′-radiolabeled PKR mRNA (25 nM; 1250 cpm per assay) and 2-5A–synthetase mRNA (25 nM; 1365 cpm per assay). Homogenous, recombinant human 2-5A–dependent RNase (5 ng) was added to a final volume of 20 μl, and incubations were at 37°C for 15 min. Reactions were terminated with 10 μl of formamide stop buffer (U.S. Biochemicals). RNA was analyzed on 8% polyacrylamide–8 M urea gels (30 cm by 40 cm by 0.04 cm).
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28. Chimeric oligonucleotides (2 μM per treatment) were added to cultures of HeLa cells at 0, 12, 24, 36, 48, 60, and 72 hours. The poly(I):poly(C) (25 μg/ml) treatment was for an additional 12 hours of incubation (added at 72 hours). Human recombinant TNF-α was added at a final concentration of 50 ng/ml for 2 hours after the oligonucleotide treatment at 72 hours. Nuclear pellets from control and chimeric oligonucleotide-treated HeLa cells were resuspended in 50 μl of nuclear extract buffer [20 mM tris (pH 8.0), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, and 25% glycerol] and used in the EMSA. For each EMSA, 1 μg of nuclear extract was incubated with 30,000 cpm of 5′-^{32}P–labeled PRDII DNA oligonucleotide for 20 min at room temperature in EMSA binding buffer [8 mM Hepes (pH 7.0), 8% glycerol, 20 mM KCl, 4 mM $MgCl_2$, 1 mM Na_3PO_4, 0.2 mM EDTA, and 0.5 μg of poly(dI):poly(dC)]. The PRDII sequence from the human interferon β gene (−66 to −55) is 5′-GTGGGAAATTCC-3′. The antibody to the p50 subunit of NF-κB (Santa Cruz Biotechnology, Santa Cruz, CA) is an affinity-purified rabbit polyclonal antibody. The binding reactions were analyzed after electrophoresis on a 4% polyacrylamide gel in 0.5× tris-borate EDTA (TBE) running buffer. An autoradiogram of the dried gel was prepared.
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