20. The localized contributions to α are due to phase transitions. The functional form that is assumed is $A_1 \exp[(z - z_1)/\sigma]^2$ with z_1 being 0.65 and 0.80, respectively, for the spinel to perovskite and the olivine to spinel phase changes and $\sigma = 0.02$, which corresponds to a phase transition width of 40 km. The constant A_1 is given in (*6, 7, 11, 12*) and depends on the density change across the transition, the Clapeyron slope, background thermal expansivity, gravity, mean density, and the depth of the mantle. For the olivine-to-spinel and spinel-to-perovskite transitions, the values of A_1 that we used were, respectively, 5 and −5. The sign of the Clapeyron slope determines the degree of local enhancement or decrease of the overall thermal expansivity. This localized α is regarded as a simplification because the effects of phase boundary distortion and change in width of the transition zone are not accounted for.
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23. The magnitudes of the Clapeyron slopes for the olivine-to-spinel and spinel-to-perovskite transitions are between 2.5 and 3.0 M Pa K^{-1} (*21, 22*). Phase changes are assumed to occur under equilibrium situations, and sluggish kinetic effects in cold descending flows are not considered.
24. There are many ways to parameterize the depth variations of mantle viscosity. An exponential dependence is based on the effect of activation volume in the mantle-creep laws [see, for example, G. Ranalli, *Rheology of the Earth* (Allen and Unwin, London, 1987)].
25. The phonon contribution to the thermal conductivity goes as ρ^4 [D. L. Anderson, *Phys. Earth Planet. Inter.* **45**, 307 (1987)].
26. S. Balachandar and D. A. Yuen, *J. Comput. Phys.*, in press. Periodic boundary conditions are imposed along the sides, and the boundary conditions at the top and bottom are stress free, impermeable, and at different constant temperatures. There is no internal heating. We used a Fourier-Chebyshev spectral expansion with 70 grid points in the vertical and 100 in the horizontal direction. We used a coordinate stretching [A. Bayliss and E. Turkel, *J. Comput. Phys.* **101**, 349 (1992)] in the vertical direction to better resolve the local dynamics. Examination of the spectral decay of the temperature and velocity components in the vertical and two horizontal directions show decay over several orders in magnitude, which indicates adequate spatial resolution of the smallest relevant length scales in all three directions. The time step of about 10^{-6} that corresponds to CFL numbers of 0.3 to 0.4 is much smaller than the smallest time scales of the flow.
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34. We thank V. Steinbach, W. Zhao, R. Boehler, U. R. Christensen, and P. Tackley for discussions. This research has been supported by the Geochemistry program at National Science Foundation, National Aeronautics and Space Administration, and the von Humboldt Stiftung of Germany. S.H. was the recipient of a visiting scholar grant from the Minnesota Supercomputer Institute.

4 November 1992; accepted 17 December 1992

Toward a Model for the Interaction Between Elongation Factor Tu and the Ribosome

Albert Weijland and Andrea Parmeggiani

In the elongation cycle of bacterial protein synthesis the interaction between elongation factor–Tu (EF-Tu)·guanosine triphosphate (GTP), aminoacyl–transfer RNA (aa-tRNA), and messenger RNA–programmed ribosomes is associated with the hydrolysis of GTP. This interaction determines the selection of the proper aa-tRNA for incorporation into the polypeptide. In the canonical scheme, one molecule of GTP is hydrolyzed in the EF-Tu–dependent binding of aa-tRNA to the ribosome, and a second molecule is hydrolyzed in the elongation factor–G (EF-G)–mediated translocation of the polypeptide from the ribosomal A site to the P site. Substitution of Asp^{138} with Asn in EF-Tu changed the substrate specificity from GTP to xanthosine triphosphate and demonstrated that the EF-Tu–mediated reactions involved the hydrolysis of two nucleotide triphosphates for each Phe incorporated. This stoichiometry of two is associated with the binding of the correct aa-tRNA to the ribosome.

Elongation factor–Tu (EF-Tu) of *Escherichia coli* is a guanine nucleotide–binding protein that functions as the carrier of aminoacyl-tRNA (aa-tRNA) to the ribosome in protein synthesis (*1*). In the past 25 years the stoichiometry of guanosine triphosphate (GTP) hydrolysis and peptide bond formation has been investigated repeatedly, but despite these efforts no conclusive results have been obtained (*2–4*). A stoichiometry of two GTP molecules hydrolyzed was derived from the evidence that EF-Tu and elongation factor–G (EF-G) each bind one molecule of GTP and that EF-Tu, GTP, and aa-tRNA form a trimeric complex. One of the GTP molecules hydrolyzed was associated with the EF-Tu–dependent binding of aa-tRNA to the ribosome prior to peptide bond formation, the other with the EF-G–mediated translocation of the polypeptide chain from the ribosomal A site to the P site, which occurs after the incorporation of a new amino acid residue. Higher stoichiometries obtained from a complete translation system were attributed to guanosine triphosphatase (GTPase) activities uncoupled from protein biosynthesis (*4*). Recently, Ehrenberg *et al.* (*5*) reopened this problem by proposing on the basis of kinetic studies that two molecules of GTP are hydrolyzed by EF-Tu for each phenylalanine incorporated. Accordingly, the carrier of aa-tRNA to the ribosomes would not be a trimeric complex but a pentameric complex containing two EF-Tu molecules. Intermolecular interactions of EF-Tu had already been suggested on the basis of genetic (*6*) and biochemical evidence (*7*), but only for a combination of two EF-Tu mutants. The proposed pentameric model remains controversial. Biochemical and physical-chemical experiments have recently supported the existence of a trimeric complex (*8*).

In order to assess in the complete elongation cycle the precise stoichiometry of the EF-Tu·aa-tRNA·ribosome interaction, we selected a method that avoids some of the inherent difficulties—that both elongation factors are GTPases, that the EF-G GTPase activity is stimulated by ribosomes in the absence of protein synthesis, and that even highly purified ribosomal preparations include nonspecific nucleotidases. Protein synthesis is complex even in a simplified in vitro system, and determination of stoichiometry requires correction factors that are difficult to measure accurately. Therefore, we distinguished EF-Tu–dependent GTP hydrolysis from EF-G and other ribosomal GTPases with a mutant EF-Tu in which substitution of Asp^{138} with Asn (EF-Tu D138N) changed the substrate specificity from GTP to xanthosine triphosphate (XTP). This change was predicted from the three-dimensional model of EF-Tu (or the related model of H-*ras* p21) in which Asp^{138} (or Asp^{119} in p21) interacts with the exocyclic 2-amino group of the purine ring of GTP (*9*). Hwang and Miller, using a maxi-cell system have reported that EF-Tu D138N forms a stable complex with XTP, aa-tRNA, and ribosomes (*10*). Because maxicells yield only small amounts of EF-Tu D138N and these are contaminated by the wild-type elongation factor, we constructed and expressed the plasmid-borne EF-Tu D138N in *E. coli* and purified the protein product to homogeneity, completely free from EF-Tu wild type (*11*).

In the presence of XTP purified EF-Tu

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D138N had the same kinetics (Fig. 1A) and accuracy in polyphenylalanine synthesis as did EF-Tu wild type in the presence of GTP, and the affinities of the mutant for XTP and XDP were the same as that of EF-Tu wild type for GTP and GDP. Like the intrinsic GTPase activity of the wild-type factor, the intrinsic xanthosine triphosphatase (XTPase) activity of EF-Tu D138N was extremely low. The specificity of EF-Tu D138N for XTP was high, and thus its elongation activity was dependent on XTP, even if GTP (needed for EF-G activity) and adenosine triphosphate (ATP; for phenylalanyl-tRNA synthetase activity) were present. Moreover, XTPase activity appeared to be absent in the complete system lacking EF-Tu D138N, whereas there was considerable GTPase activity that was attributable to uncoupled EF-G–dependent activity (Fig. 1B).

The kinetic study of polyphenylalanine synthesis and of the associated hydrolysis of [γ-^{32}P]XTP demonstrated unequivocally that two molecules of XTP were hydrolyzed for each phenylalanine incorporated (Fig. 2). The stoichiometry was not changed by varying the concentration of XTP (2.02 ± 0.04; n = 16) (Fig. 3A) or EF-Tu (2.06 ± 0.04; n = 9) (Fig. 3B). We also tested the effect of magnesium, which can influence elongation rate, accuracy, and uncoupled GTPase in protein synthesis (*4*, *12*); no variation of the stoichiometry was obtained in the range for optimal polyphenylalanine synthesis (5 to 10 mM $MgCl_2$). The average length of the polypeptide chains in these experiments was at least 20 amino acid residues because this length was needed for a precise analysis of the stoichiometries under different conditions (*13*). Two XTP molecules were hydrolyzed by EF-Tu for each Phe-tRNA^{Phe} bound to the poly(U)·ribosome complex in the absence of EF-G whether the ribosomal P site was occupied by tRNA^{Phe} (Table 1) or by *N*-acetylPhe-tRNA^{Phe}; in the former instance no peptide bond formation occurred.

Because EF-G had been determined to

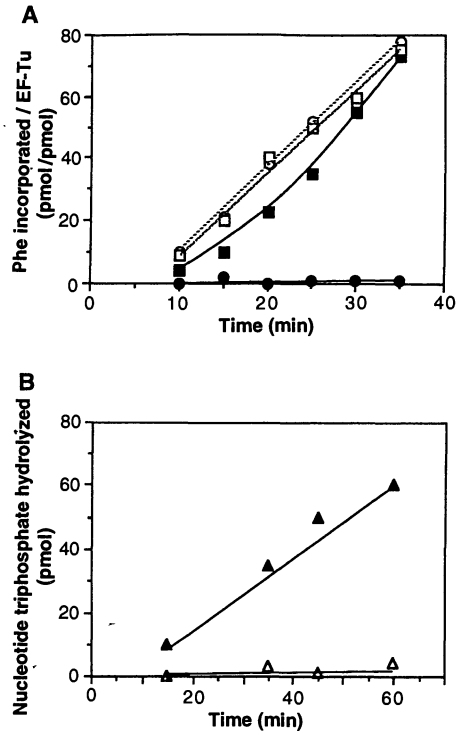

Fig. 1. Properties of the EF-Tu D138N/XTP system: dependence on XTP of polyphenylalanine synthesis with EF-Tu D138N (**A**) and absence of nonspecific XTPases in the complete system lacking EF-Tu D138N (**B**). (A) The reaction mixture (400 μl) contained standard buffer [50 mM tris-HCl (pH 7.6), 70 mM NH_4Cl, 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol], 0.025 μM EF-Tu D138N (solid symbols) or EF-Tu wild type (open symbols), with 100 μM XTP (squares) or without XTP (circles), 1 mM ATP, 100 μM GTP, 0.2 μM EF-G, 0.1 μM EF-Ts, 130 μg of poly(U), 0.5 μM ribosomes, 6 μM [^{14}C]phenylalanine (0.05 Ci/mmol), 4 μM tRNA^{Phe}, and 0.015 μM purified phenylalanyl-tRNA synthetase. The reaction was incubated at 30°C and initiated with Phe-tRNA^{Phe}. Samples (50 μl) were withdrawn and the polyphenylalanine synthesized was measured by the trichloroacetic acid method (*11*). Blanks obtained with the complete system minus EF-Tu D138N were performed for each point. The values subtracted were 4 to 5 pmol of polyphenylalanine synthesized for each pmol of EF-Tu. In (B) the polyphenylalanine reaction mixture lacking EF-Tu was the same as in (A) and also contained 50 μM [γ-^{32}P]GTP (0.15 Ci/mmol) or [γ-^{32}P]XTP (0.1 Ci/mmol). Samples (50 μl) were withdrawn, and the $^{32}P_i$ liberated from GTP (▲) or XTP (△) at 30°C was measured (*12*). Blanks were measured in the absence of EF-G and ribosomes. The values subtracted were 6 to 8 pmol of $^{32}P_i$ in the XTP reactions and 13 to 18 pmol in the GTP reactions. The tRNA^{Phe} was 60 to 70% pure. Highly purified NH_4Cl-washed ribosomes, EF-Tu, EF-Ts, EF-G, and phenylalanyl-tRNA synthetase were prepared as described (*18*).

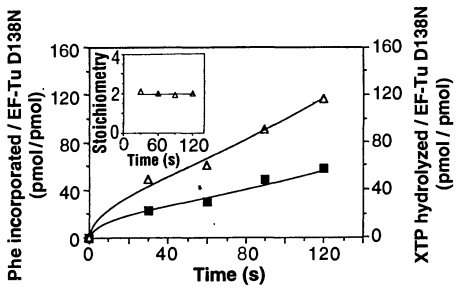

Fig. 2. Stoichiometry of XTP hydrolysis and peptide bond formation. The mRNA-programmed ribosomes carrying peptidyl-tRNA in the P site were prepared by first incubating for 40 min at 0°C 120 pmol of ribosomes in 120 μl of standard buffer with 70 μg of poly(U) and 140 pmol of *N*-acetylPhe-tRNA^{Phe}. The reaction (30 μl) was then incubated at 30°C and was started by the addition of an equal volume of a mixture containing 2 mM ATP, 200 μM GTP, 0.05 μM EF-Ts, 16 μM [γ-^{32}P]XTP (0.3 Ci/mmol), 0.01 μM EF-Tu D138N, 5 μM tRNA^{Phe}, 8 μM [^{3}H]phenylalanine (0.4 Ci/mmol), and 0.015 μM purified phenylalanyl-tRNA synthetase and 0.1 μM EF-G. Samples (7 μl) were removed at intervals. A sample lacking EF-Tu D138N was used as control. The subtracted values were 20, 22, 23, and 25 pmol of $^{32}P_i$ and 2.6, 1.7, 3.0, and 1.9 pmol of phenylalanine incorporated for each pmol of mutant EF-Tu, respectively. A second control consisting of the complete system minus phenylalanyl-tRNA synthetase and ATP was used to test the extent of the intrinsic XTPase activity, which was negligible. Phenylalanine incorporation (■) and $^{32}P_i$ liberated (△) were measured by the TCA and charcoal methods, respectively (*13*). Inset is the ratio (△) of XTP consumption to phenylalanine incorporation.

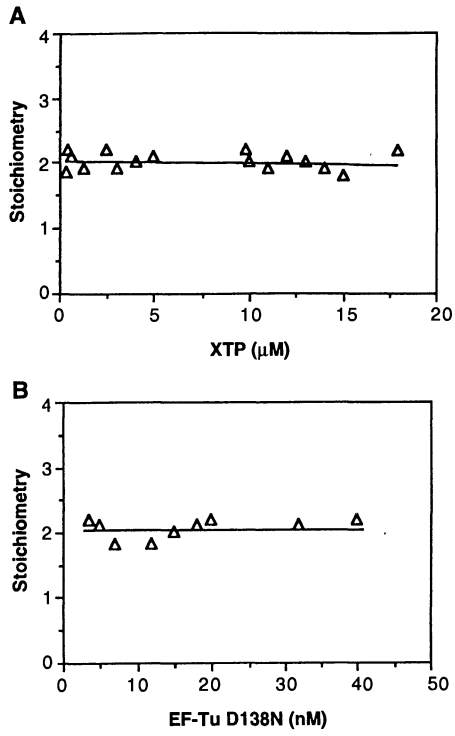

Fig. 3. Ratio (△) of XTP hydrolysis to peptide bond formation as a function of XTP (**A**) and EF-Tu D138N (**B**) concentration. (A) The reaction mixture was the same as in Fig. 1A except that 0.01 μM EF-Tu D138N, 6 μM [^{3}H]phenylalanine (0.45 Ci/mmol), and 5 μM [γ-^{32}P]XTP (0.3 Ci/mmol) were used. (B) The reaction mixture was the same as in Fig. 2 with poly(U)-programmed ribosomes carrying *N*-acetylPhe-tRNA^{Phe} in the P site. The stoichiometry at 30°C was determined over a period of 25 min (A) or 2 min (B). Polyphenylalanine synthesis was measured by nitrocellulose binding, and [γ-^{32}P]XTP hydrolysis was measured by the charcoal method (*13*). The blank values subtracted were in the same range as those for the experiments in Fig. 2.

hydrolyze one molecule of GTP for each polypeptidyl-tRNA translocated (*3*, *14*), these results imply that the total number of nucleotide triphosphates hydrolyzed by EF-Tu and EF-G should be three for each elongation cycle. Accordingly, at magnesium concentrations of 5 to 9 mM, when the uncoupled EF-G GTPase activity is low (*4*), one molecule of GTP and two molecules of XTP were hydrolyzed with the EF-Tu D138N–XTP system for each phenylalanine incorporated into the polypeptide (Fig. 4). At magnesium concentrations higher than 9 mM the uncoupled GTP hydrolysis of EF-G predominated.

The first point to consider is why EF-Tu–dependent aa-tRNA interaction with the ribosome requires the hydrolysis of two nucleotide triphosphate molecules. The constant stoichiometry of 2 (Figs. 2 and 3) makes a futile reaction improbable. Because both hydrolyses are associated with binding of aa-tRNA to the ribosome, we propose that they contribute to the selection of the correct aa-tRNA. At low XTP concentrations, which limited the regeneration of the complex between EF-Tu and aa-tRNA, the amount of XTP hydrolyzed and aa-tRNA bound to the ribosome increased from Leu-$tRNA_2^{Leu}$ (containing the anticodon GAG) to Leu-$tRNA_4^{Leu}$ (NAA) and Phe-$tRNA^{Phe}$ (GAA), while the corresponding stoichiometry decreased from 7 to 5 to 2, respectively (Table 1). Thus in the presence of aa-tRNAs whose anticodons bound with different affinities to the poly(U) template (*15*), the ratio of XTP hydrolysis to aa-tRNA bound to ribosome changed as expected for a proofreading mechanism (*16*). The possibility that the two GTPase reactions take place sequentially would allow the ribosome to build up a higher level of accuracy, according to the model proposed by Hopfield and Ninio (*17*).

The second point to consider is how to reconcile a stoichiometry of two GTP molecules hydrolyzed by EF-Tu for each peptide bond formed with the experiments supporting a trimeric complex as the carrier of aa-tRNA to the ribosome (*8*). Even though our results are compatible with the participation of two EF-Tu and two GTP molecules in a pentameric complex with aa-tRNA, as suggested by Ehrenberg *et al.* (*5*), the mechanism of how two EF-Tu molecules can interact with the ribosome during the elongation cycle is still not known precisely. It is possible that only one EF-Tu·GTP is part of a stable complex with aa-tRNA and that it may interact weakly with a second EF-Tu·GTP that is also in contact with the ribosome. The hydrolysis of the first GTP that occurs on codon-anticodon interaction would induce a conformational change of the ribosome that would trigger the GTPase activity of the second EF-Tu. In conclusion, the results obtained with the EF-Tu D138N–XTP system provide experimental evidence that the mechanism of EF-Tu–mediated amino acid incorporation is controlled by two high-energy driven steps that involve the participation of two EF-Tu molecules in the selection of the correct aa-tRNA.

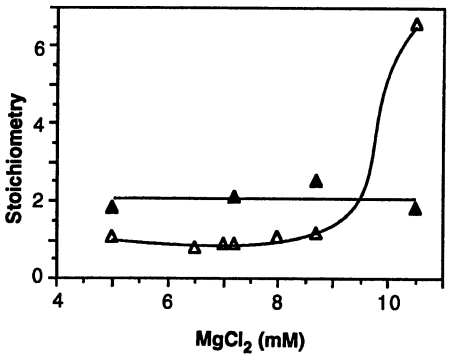

Fig. 4. Stoichiometry of XTP or GTP hydrolysis and polyphenylalanine synthesis with EF-Tu D138N as a function of $MgCl_2$ concentration. For each $MgCl_2$ concentration the reaction mixture (28 μl) was the same as in Fig. 1 except for 0.01 μM EF-G, 4 μM XTP, and 20 μM [γ-^{32}P]GTP (0.3 Ci/mmol) (open symbols) or 0.01 μM EF-G, 4 μM [γ-^{32}P]XTP (0.3 Ci/mmol), and 20 μM GTP (solid symbols). The reaction was initiated with Phe-$tRNA^{Phe}$ at 30°C. After a 20-min incubation two 13-μl samples were withdrawn to determine phenylalanine incorporation by nitrocellulose binding and $^{32}P_i$ liberated by the charcoal method (*13*); Blanks were measured for the complete system in the absence of EF-G (open symbols) or in the absence of EF-Tu (solid symbols) at each point. The blank values subtracted were in the same range of those for the experiments in Fig. 2.

Table 1. Stoichiometry of XTP hydrolysis and EF-Tu D138N–dependent binding of cognate and noncognate aa-tRNAs to the ribosomal A site of poly(U)-programmed ribosomes. The final reaction mixture was prepared by adding poly(U)-programmed ribosomes [100 μl of standard buffer containing 25 pmol of ribosomes, 14 μg of poly(U), and 60 pmol of $tRNA^{Phe}$ to occupy the ribosomal P site] to the complex formed between EF-Tu and aa-tRNAs [50 μl of standard buffer containing 4 pmol of [γ-^{32}P]XTP (0.32 Ci/mmol), 5 pmol of EF-Tu D138N, 5 pmol of EF-Ts, 5 pmol of aa-tRNA: [^{3}H]Phe-$tRNA^{Phe}$ (0.32 Ci/mmol), [^{3}H]Leu-$tRNA_2^{Leu}$ (1 Ci/mmol), or [^{3}H]Leu-$tRNA_4^{Leu}$ (1 Ci/mmol)]. After 3 min at 30°C, two 50 μl-samples were withdrawn. The first was pipetted onto a nitrocellulose filter to determine the amount of tritiated phenylalanine or leucine bound to ribosomes, and the second was used to measure the amount of $^{32}P_i$ liberated (*13*). In all experiments controls in the absence of EF-Tu D138N were performed; the blank values subtracted are indicated in parentheses. The $tRNA_2^{Leu}$ and $tRNA_4^{Leu}$ were obtained from Subriden RNA (Rolling Bay, Washington). Values are mean ± SEM (n = 4).

aa-tRNA	aa-tRNA bound (pmol)	XTP hydrolyzed (pmol)
Phe-$tRNA^{Phe}$	1.0 ± 0.04 (0.2)	2.0 ± 0.03 (0.1)
Leu-$tRNA_4^{Leu}$	0.28 ± 0.02 (0.06)	1.4 ± 0.04 (0.1)
Leu-$tRNA_2^{Leu}$	0.13 ± 0.01 (0.07)	0.9 ± 0.04 (0.1)

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13. The stoichiometry did not vary when highly purified NH_4Cl-washed ribosomes from different preparations or ribosomes purified from different *E. coli* strains [MRE600, B, or LBE1001] were used. In order to avoid partially active ribosomes that were defective in peptidyl transferase, we determined the amount of phenylalanine incorporated into the polypeptide chain either by precipitation with trichloroacetic acid (TCA) or by retention on nitrocellulose filters. The first method permitted the measurement of polypeptide chains longer than three to four amino acid residues. Paper disks (Schleicher & Schull, Rundfilter 2316/8, 25 mm) carrying the samples were washed with 10% cold TCA, boiled

for 10 min in 5% TCA, and washed for 1 min each in 5% cold TCA, ethanol, ethanol-ether (1:1, v/v), and ether. They were dried, and the retained radioactivity was measured. The second method also detected oligopeptides of less than 3 to 4 residues and aa-tRNA bound to ribosomes. Nitrocellulose filters (Sartorius, Long Island, NY; SM 11306, 0.45 μM) were washed twice with 2 ml of standard buffer and dried, and the radioactivity retained was measured. In these experiments the average chain length was at least 20 residues; the stoichiometries obtained with these methods were similar (Figs. 2 and 3). The chain length was calculated from the $^{3}H/^{14}C$ ratio obtained with poly(U)-programmed ribosomes that bound *N*-acetyl[^{14}C]Phe-tRNA^{Phe} in the P site and incorporated [^{3}H]Phe-tRNA^{Phe} in the elongation steps. Inorganic phosphate (P_i) liberated from XTP or GTP was measured by the charcoal method. The reaction, stopped with one volume of 1 M $HClO_4$, 20 mM KH_2PO_4 was diluted tenfold with a 6% charcoal solution in 1 N HCl and centrifuged for 10 min, and the radioactivity in an aliquot was determined (*18*). [γ-^{32}P]XTP was prepared according to I. M. Glynn and J. B. Chappell [*Biochem. J.* **90**, 147 (1964)].

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19. Supported by grants of the Ligue Nationale Française Contre le Cancer, the Association pour la Recherche contre le Cancer, and the Institut National de la Santé et de la Recherche Médicale. We thank A. L. Haenni, P. H. Anborgh, K. Harmark, and J. Jonak, for reviewing the manuscript, and S. Kirillov (B. P. Konstantinov Petersburg Nuclear Physics Institute, Gatchina) for providing highly purified ribosomes from *E. coli* MRE600.

14 August 1992; accepted 15 December 1992

RBP9-27 protein

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                                                    40
MHKEEHEVAV LGAPPSTILP RSTVINIHSE TSVPDHVVWS

                                                    80
LFNTLFLNWC CLGFIAFAYS VKSRDRKMVG DVTGAQAYAS

                                                   125
TAKCLNIWAL ILGILMTIGF ILSLVFGSVT VYHIMLQIIQ EKRGY
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GST–RBP9-27 hybrid protein

```
GST--CTG GTT CCG CGT GGA TCC CCG GGC CAG AAG ATG--RBP927
     leu val pro arg gly ser pro gly gln lys met
                    ↑
     Thrombin cleavage site
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Fig. 1. Amino acid sequences (*27*) of RBP9-27 and of the GST–RBP9-27 recombinant protein expressed in bacteria. The first 28 amino acids of RBP9-27 (underlined) are missing from the β-gal–RBP9-27 hybrid protein produced in λ clone 11. The sequence of the insert in clone 11 begins with Ser^{29} and consists of the 97 COOH-terminal amino acids of the RBP9-27 protein. For bacterial expression, 9-27 cDNA was linked to the GST gene and expressed in pGEX-2T. The hybrid protein was purified and digested with thrombin, resulting in RBP9-27 with six additional amino acids at the NH_2-terminus.

Inhibition of Rev-Mediated HIV-1 Expression by an RNA Binding Protein Encoded by the Interferon-Inducible 9-27 Gene

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Interferon inhibits expression of human immunodeficiency virus type–1 (HIV-1) through unknown mechanisms. A gene inducible by interferon-α (IFN-α) and interferon-γ (IFN-γ) was isolated by screening of a human complementary DNA library for proteins binding to the Rev-responsive element (RRE) of HIV-1. The product of this gene, RBP9-27, was shown to bind RNA in vitro and to inhibit HIV-1 expression after transfection into human cells. RBP9-27 primarily inhibited Rev-dependent posttranscriptional steps of viral gene expression. Thus, RBP9-27 is a cellular factor that antagonizes Rev function. These results suggest an interferon-induced antiviral mechanism operating through the induction of RNA binding proteins such as RBP9-27. Elucidation of RBP9-27 function may lead to a better understanding of the mechanism of interferon action during HIV-1 infection.

Interferons induce a large number of genes in either a direct or an indirect fashion (*1, 2*). The products of these genes either singly or coordinately mediate the antiviral, growth inhibitory, or immunoregulatory activities of interferons. The antiviral effects of interferons against HIV-1 are well established (*3*). Variable amounts of interferon production have been reported in HIV-infected individuals (*4, 5*). Clinical trials of interferon in acquired immunodeficiency syndrome (AIDS) patients are being conducted on the basis of the inhibitory effects of interferon in tissue culture (*6*). Further understanding of the mechanisms of interferon-mediated HIV-1 inhibition may lead to the optimization of the antiviral effects of interferon.

During a screening for cellular proteins that bind to the RRE of HIV-1, we identified an RNA binding protein that inhibits HIV-1 expression. RRE is the site of interaction of the HIV-1 protein Rev with the viral mRNA (*7*). RRE is composed of approximately 210 nucleotides; is located in the *env* coding region of HIV-1, and displays a complex structure important for Rev binding and function (*8, 9*). Rev is required for the expression of structural proteins and thus viral particle formation. Rev protein promotes HIV-1 expression by participating in complex posttranscriptional regulatory steps that involve both positive and negative elements. Rev counteracts the down-regulatory effects of cis-acting HIV-1 sequences (*10–12*) that decrease mRNA transport, stability, and use (*9, 10, 13, 14*). Additional cellular factors are important for Rev function (*15, 16*).

To identify cellular proteins that bind to RRE RNA, we used a 330-nucleotide RRE RNA probe, RRE330 (*15*), uniformly labeled with [^{32}P]uridine triphosphate (UTP) to screen a λ-ZAP XR-II cDNA expression library derived from RNA of the human U937 monocyte cell line (*17*). Twelve recombinant phages were isolated that expressed proteins binding to the RRE probe and not to other RNAs, such as the Tat binding site (TAR, known to form a strong secondary structure) or a globin RNA fragment. We also assessed the binding of the λ clones to different mutated forms of RRE (RREΔ345, RREΔ12s, and RRE220) (*15, 17*). One clone (clone 11) bound to the intact RRE (RRE330), RRE220, and RREΔ12s but did not bind to RREΔ345. This suggested that the recombinant protein produced by clone 11 bound to the region of the RRE containing hairpin loops 3, 4, and 5. The 850-nucleotide cDNA insert of clone 11 contained an open reading frame of 97 amino acids, positioned in-frame with β-galactosidase in λ-ZAP (Fig. 1). A computer search of the GenBank–European Molecular Biology Laboratory (EMBL) database revealed complete identity with the human interferon-inducible gene 9-27 (*18*). The 9-27 gene encodes a protein of 125 amino acids. The cDNA insert in clone 11 encodes the COOH-terminal 97–amino acid residues of this protein, starting at Ser^{29} (Fig. 1). Because our experiments show that the product of the 9-27 gene is an RNA binding protein, we refer to it as RBP9-27.

To express the protein in bacteria, we generated a cDNA fragment of 555 bp after

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