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Interferon action: Two distinct pathways for inhibition of protein synthesis by double-stranded RNA

(2′,5′-oligoadenylate/protein kinase/endonuclease/initiation factor eIF-2)

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ABSTRACT **Double-stranded RNA inhibits protein synthesis in at least two ways. It activates a protein kinase that blocks peptide chain initiation by phosphorylating the peptide chain initiation factor eIF-2 and also activates an endonuclease that inactivates different mRNAs at different rates. The protein kinase and the endonuclease have been partially purified from interferon-treated Ehrlich ascites tumor cells. The 2′,5′-oligoadenylates [pppA(2′p5′A)$_n$], found earlier to be mediators in the activation of the endonuclease by double-stranded RNA, are not mediators in the activation of the protein kinase by double-stranded RNA.**

Interferons are glycoproteins that are synthesized in various vertebrate cells after virus infection. They are secreted, they interact with other cells, and they convert these cells into the antiviral state in which virus replication is impaired (1). The effect of interferon treatment of cells is manifested in their extracts in various ways (2). Some of these depend on the addition of double-stranded (ds) RNA to the extract. Thus, extracts from interferon-treated L cells or HeLa cells are more susceptible to inhibition of protein synthesis by dsRNA than extracts from control cells (3, 4). Furthermore, dsRNA and ATP, added to extracts from interferon-treated Ehrlich ascites tumor (EAT) or HeLa cells, activate an endonuclease, but less so if added to extracts from control cells (5–7). We have purified this endonuclease about 80-fold. The "nuclease activators" that mediate the activation by dsRNA and ATP are compounds of general structure pppA(2′p5′A)$_n$ (8, 9). These compounds were discovered by Kerr and coworkers as inhibitors of protein synthesis and were found to be synthesized in extracts of interferon-treated mouse L cells (10, 11), EAT cells (8), chicken embryo cells (12), and in reticulocyte lysates (13) if supplemented with dsRNA and ATP. Finally, dsRNA and ATP also activate, in extracts of interferon-treated EAT (14), L (10), and HeLa (4) cells and reticulocyte lysates (15), one or more protein kinase systems that phosphorylate at least two proteins: P1 (molecular weight 67,000) and P2 (molecular weight given variously as 35,000–37,000). P2 has been tentatively identified as the small subunit of the peptide chain initiation factor eIF-2. This identification was based on the mobility of the protein in sodium dodecyl sulfate ($NaDodSO_4$) gels and on the analogy with the phosphorylation of eIF-2 in response to dsRNA in rabbit reticulocyte lysates (16–18). We have purified a latent protein kinase system from extracts of interferon-treated EAT cells about 1000-fold (19). The system can be activated by incubation with dsRNA and ATP and the activated system phosphorylates a *Drosophila* histone H1 preparation even after the degradation of dsRNA by RNase III. The fraction containing the kinase system was called PC-II$_{INT}$. A fraction purified in the same way as PC-II$_{INT}$ but from cells not treated with interferon (designated as PC-II$_C$) does not phosphorylate the *Drosophila* histone H1 preparation even after incubation with dsRNA and ATP (19).

We show here that PC-II$_{INT}$ is a potent inhibitor of protein synthesis in reticulocyte lysates, but only if activated by incubation with dsRNA and ATP. It acts by phosphorylating and thereby inactivating the small subunit of the peptide chain initiation factor eIF-2 (ref. 20). The compounds pppA(2′p5′A)$_n$ are apparently not mediators in the activation of the protein kinase system by dsRNA and ATP. These results were presented at the European Molecular Biology Organization–Federation of European Biochemical Societies, meeting on "New Aspects of Interferon Research" (Warwick, England, April 1978).

MATERIALS AND METHODS

EAT cells were grown and treated with interferon as described (8). PC-II$_{INT}$ is a protein fraction purified from the high-salt wash of ribosomes from EAT cells that have been treated with 650 reference units of interferon (8) (specific activity 2×10^7 units/mg of protein) per ml of culture for 18 hr (19). PC-II$_{INT}$ was purified about 1000-fold for dsRNA-activated protein kinase activity, with [γ-^{32}P]ATP and a *Drosophila* histone H1 preparation as substrates. PC-II$_C$ is a protein fraction purified in the same way as PC-II$_{INT}$ but from EAT cells not treated with interferon (19).

PC-II$_{INT}$ was activated by preincubation with dsRNA in the following way. PC-II$_{INT}$ (0.55 mg/ml) or PC-II$_C$ (2.2 mg/ml) was preincubated in 33 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/6 mM 2-mercaptoethanol/0.1 mM ATP/3% glycerol (vol/vol) with or without 1.7 μg of poly(I)·poly(C) per ml at 30°C for 10 min. The standard reticulocyte lysate protein-synthesizing system was prepared and samples were processed as described (16) except that the reaction mixtures were supplemented with 1.6 mM glucose. 6B ribosomes were prepared by passing reticulocyte lysate through Sepharose 6B (15). The mRNA-dependent reticulocyte lysate protein-synthesizing system was prepared and used according to Pelham and Jackson (21). The nuclease-treated lysate was supplemented with 100 μg of mouse liver tRNA per ml. pppA(2′p5′A)$_n$ was synthesized (as indicated in the figure legends) either by incubating dsRNA and ATP with DE-1$_{INT}$ [a protein fraction partially purified from interferon-treated EAT cells (8)] or by the poly(I)·poly(C) column procedure (22) with an extract from interferon-treated EAT cells. The pppA(2′p5′A)$_n$ was freed of ATP by chromatography on DEAE-cellulose (22). Concentrations of pppA(2′p5′A)$_n$ were based on the amount of ^{32}P incorporated from [α-^{32}P]ATP and the assumption that the mixture consists of pppA2′p5′A2′p5′A only. A detailed characterization of pppA(2′p5′A)$_n$ will be reported elsewhere.

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Abbreviations: ds, double-stranded; EAT, Ehrlich ascites tumor; eIF-2, initiation factor 2; $NaDodSO_4$, sodium dodecyl sulfate.

RESULTS

Activated PC-II$_{INT}$ Inhibits Protein Synthesis and Inhibition Is Overcome by Added eIF-2. The data in Fig. 1*A* reveal that, if activated by incubation with dsRNA and ATP, PC-II$_{INT}$ inhibits endogenous protein synthesis in a reticulocyte lysate. PC-II$_{INT}$ and ATP preincubated without dsRNA or PC-II$_C$ and ATP preincubated with or without dsRNA have little or no such effect. The inhibitory effect of activated PC-II$_{INT}$ is lost upon heating at 95°C for 2 min (data not shown). The inhibition of protein synthesis by activated PC-II$_{INT}$ is manifested after 5 min of incubation (Fig. 1*B*). The impairment can be overcome by addition of peptide chain initiation factor eIF-2 (Fig. 1*C*).

In the above experiments the reticulocyte lysates were supplemented with dsRNA at a high concentration (20 μg/ml) to prevent inhibition resulting from the carry-over of dsRNA from the preincubations. This supplementation caused little or no inhibition of endogenous protein synthesis under the conditions and in the time span of the experiments (Fig. 1*B*). The fact that, at least if tested in short incubations, endogenous protein synthesis in reticulocyte lysates is inhibited by dsRNA at low but not at high concentrations has been reported previously (15, 17, 24). Activated PC-II$_{INT}$ (but not PC-II$_C$ similarly preincubated) also inhibits mRNA translation in an extract from EAT cells (not shown).

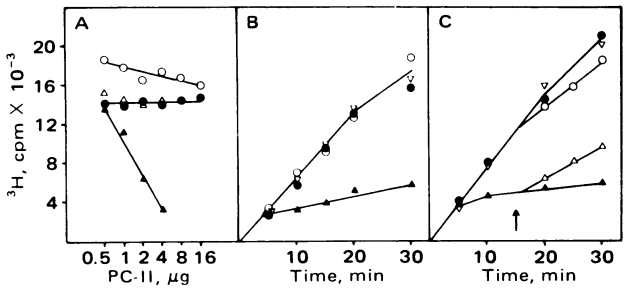

FIG. 1. Inhibition of endogenous protein synthesis in a rabbit reticulocyte lysate by PC-II$_{INT}$ activated by preincubation with dsRNA and ATP. (*A*) Inhibition of protein synthesis by activated PC-II$_{INT}$. Various amounts of PC-II$_{INT}$ and PC-II$_C$ (μg/80 μl of reaction mixture), preincubated with or without poly(I)·poly(C), were included in standard protein-synthesizing systems containing rabbit reticulocyte lysate and 20 μg of dsRNA per ml (in the figure legends, unless otherwise stated, dsRNA was from *Penicillium chrysogenum*). Incubation mixtures contained: PC-II$_C$ preincubated without (○) or with (●) poly(I)·poly(C) or PC-II$_{INT}$ preincubated without (△) or with (▲) poly(I)·poly(C). After incubation at 30°C for 30 min, 4-μl samples were taken to determine the amount of [^{3}H]leucine (206 Ci/mol) incorporated into trichloroacetic acid-insoluble material. An incubation with no additions gave 16,000 cpm. (*B*) Time course of inhibition of protein synthesis. Two micrograms of activated PC-II$_{INT}$ (▲) or of PC-II$_C$ similarly preincubated (●) or an equal volume of water (▽) was included in standard protein-synthesizing systems (80 μl) containing reticulocyte lysate and 20 μg of dsRNA per ml. A standard protein-synthesizing system (80 μl) without any additions was also prepared (○). Incubation was at 30°C; 4-μl aliquots were taken at the times indicated for assay of protein synthesis. (*C*) Inhibition of protein synthesis by activated PC-II$_{INT}$ is overcome by addition of eIF-2. Two micrograms of activated PC-II$_{INT}$ (▲,△) or of PC-II$_C$ similarly preincubated (●,○) or an equal volume of water (▽) was included in standard protein-synthesizing systems (80 μl) containing reticulocyte lysate and 20 μg of dsRNA per ml. Incubation was at 30°C. Two of the reaction mixtures (○,△) were supplemented after 15 min (arrow) with 10 μg of eIF-2 highly purified from rabbit reticulocytes (23). Aliquots of 4 μl were taken at the times indicated for assay of protein synthesis. The slight decrease in the rate of protein synthesis in the reaction mixture with PC-II$_{INT}$, resulting from addition of the eIF-2 preparation, is thought to be due to the glycerol content of the preparation.

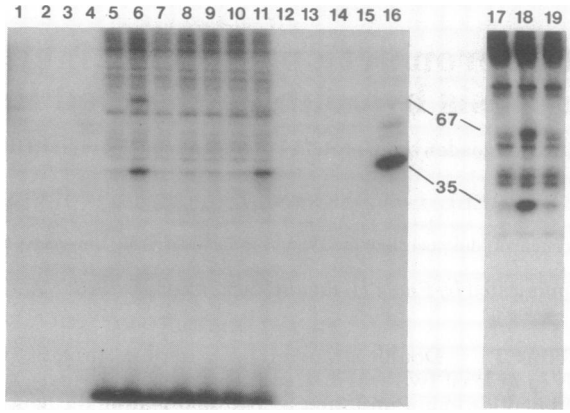

FIG. 2. PC-II$_{INT}$ contains a protein kinase that is activated by dsRNA and ATP and phosphorylates the small subunit of eIF-2. (*Left*) Dependence of eIF-2 phosphorylation on dsRNA. PC-II$_{INT}$, PC-II$_C$, or the corresponding reaction mixtures without either PC-II$_{INT}$ or PC-II$_C$ were preincubated with or without poly(I)·poly(C) (1.7 μg/ml). Then test incubation mixtures (30 μl) were assembled. These contained 3 μl of one of the preincubation mixtures (as indicated) in buffer B (20 mM Tris·HCl, pH 7.5/120 mM KCl/10 mM NaCl/1.5 mM $MgCl_2$/0.25 mM dithiothreitol) as well as 0.143 mM [γ-^{32}P]ATP (2 Ci/mmol) and substrate as indicated. Tracks 1, 8, and 13 also contained PC-II$_C$ preincubated without dsRNA; tracks 2, 9, and 14, PC-II$_C$ preincubated with dsRNA; tracks 3, 10, and 15, PC-II$_{INT}$ preincubated without dsRNA; tracks 4, 11, and 16, PC-II$_{INT}$ preincubated with dsRNA; tracks 5, 6, 7, and 12, preincubation mixture with neither dsRNA nor PC-II$_C$ nor PC-II$_{INT}$. Other additions were: tracks 5–11, 10 μg of 6B ribosomes; track 6, 50 ng of dsRNA per ml; tracks 7–11, 20 μg of dsRNA per ml; tracks 12–16, 2 μg of highly purified rabbit reticulocyte eIF-2 (23). After incubation at 30°C for 15 min the reactions were terminated by addition of $NaDodSO_4$ gel sample buffer and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. (*Right*) pppA(2′p5′A)$_n$ does not substitute for dsRNA in promoting eIF-2 phosphorylation by ribosome-associated protein kinases. Reaction mixtures (15 μl) contained 10 μg of 6B ribosomes and 130 μM [γ-^{32}P]ATP (2 Ci/mmol) in buffer B as well as the following additions: track 17, none; track 18, 70 ng of dsRNA per ml; track 19, 80 nM pppA(2′p5′A)$_n$ [prepared by the poly(I)·poly(C) column procedure]. Reaction mixtures were incubated at 30°C for 15 min and analyzed by $NaDodSO_4$ gel electrophoresis and radioautography (15). Locations on the autoradiographs of the 67,000-dalton and 35,000-dalton proteins are indicated as 67 and 35, respectively.

Activated PC-II$_{INT}$ Impairs Peptide Chain Initiation by Phosphorylating eIF-2. The finding that added eIF-2 overcomes the impairment of protein synthesis by activated PC-II$_{INT}$ raises the possibility that the impairment may be a consequence of an inactivation of eIF-2. Indeed, the treatment of reticulocyte lysate with activated PC-II$_{INT}$ results in the disaggregation of polysomes unless peptide chain elongation is blocked with anisomycin (not shown). This suggests that the inhibition of protein synthesis by activated PC-II$_{INT}$ is a consequence of an inhibition of peptide chain initiation. Furthermore, activated PC-II$_{INT}$ strongly inhibits the binding of Met-tRNA$_f$ to the 40S ribosomal subunit (not shown). This binding involves the action of eIF-2 and is inhibited in reticulocyte lysates by added dsRNA (25).

More definitive support for the hypothesis that activated PC-II$_{INT}$ inactivates eIF-2 is shown in Fig. 2. The data reveal that PC-II$_{INT}$ that has been activated by preincubation with dsRNA and ATP phosphorylates the small subunit of eIF-2 (35,000 daltons) strongly and the two larger subunits very slightly. Under no conditions was PC-II$_C$ found to phosphorylate eIF-2. The phosphorylation of the small subunit of eIF-2 by activated PC-II$_{INT}$ occurs with both eIF-2 present as a component of a crude ribosome preparation (track 11) and

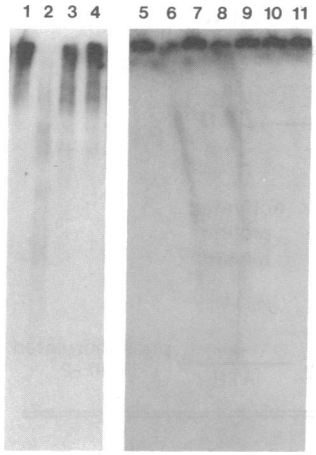

FIG. 3. (*Left*) PC-II$_{INT}$ does not generate pppA(2′p5′A)$_n$ even in presence of dsRNA. Two protein fractions (DE-1 and DE-2) were partially purified from an extract of interferon-treated EAT cells (8). Incubation of DE-1 with dsRNA and ATP gives rise to the nuclease activator pppA(2′p5′A)$_n$ (8). Tracks 1 and 2 demonstrate that fraction PA-2$_{INT}$ [a subfraction from DE-2$_{INT}$, obtained by further purification on poly(A)-Agarose, overall enrichment about 80-fold; unpublished data] contains a latent endonuclease that is activated upon incubation with the nuclease activator. The latent endonuclease in PA-2$_{INT}$ is not activated by dsRNA and ATP because it lacks the enzyme that makes pppA(2′p5′A)$_n$ (unpublished data). The endonuclease assay (8) (50 μl) in buffer C (25 mM Tris·HCl, pH 8.0/120 mM KCl/5 mM magnesium acetate/6 mM 2-mercaptoethanol) contained 0.9 μg of PA-2$_{INT}$ and 4 μg of R17 bacteriophage [^{32}P]RNA (10,000 cpm/μg). One of the reaction mixtures (track 2) was also supplemented with 35 nM pppA(2′p5′A)$_n$. After incubation at 30°C for 2 hr, the reaction mixtures were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography (8). Tracks 3 and 4 demonstrate that PC-II$_{INT}$ does not synthesize pppA(2′p5′A)$_n$ if incubated with dsRNA and ATP. PC-II$_{INT}$ (4.5 μg in a volume of 15 μl) was incubated in buffer C containing 1 mM ATP. One of the reaction mixtures (track 4) was also supplemented with 5 μg of poly(I)·poly(C) per ml. After incubation at 30°C for 2 hr, the mixtures were first heated at 100°C for 5 min, then cooled to 30°C and supplemented with 0.9 μg of PA-2$_{INT}$ and 4 μg of R17 bacteriophage [^{32}P]RNA (in a final volume of 30 μl) in buffer C. After further incubation at 30°C for 2 hr, the reaction mixtures were processed as above. (*Right*) Reticulocyte lysate contains a latent endonuclease that can be activated by dsRNA at high concentrations or by pppA(2′p5′A)$_n$. Standard reticulocyte lysate protein-synthesizing systems were prepared except that amino acids were omitted. Lysate with no added heme was used in experiments in tracks 5 and 11; lysate supplemented with 20 μM heme was used in those in tracks 6–10. Other additions were: track 6, 100 nM pppA(2′p5′A)$_n$ [prepared by the poly(I)·poly(C) column procedure]; track 7, an equivalent amount of material prepared in the same way but by using an extract from control EAT cells; track 8, 20 μg of dsRNA per ml; track 9, 50 ng of dsRNA per ml. Each reaction mixture (33 μl) was supplemented with 2 μg of R17 bacteriophage [^{32}P]RNA (120,000 cpm/μg). The mixtures were incubated at 30°C and reactions were terminated by addition of 20 μl of RNA gel sample buffer after 30 min of incubation (tracks 6–11) or no incubation (track 5). The reaction mixtures were processed as in *Left*.

with highly purified eIF-2 (98% pure) (track 16). The latter result indicates that PC-II$_{INT}$ does contain a dsRNA-activated protein kinase that phosphorylates the small subunit of eIF-2 and does not act only by activating a protein kinase otherwise latent in the crude ribosome fraction. It remains to be seen why activated PC-II$_{INT}$ does not phosphorylate the 67,000-dalton protein which is phosphorylated in crude ribosomes in the presence of dsRNA at low concentrations (tracks 6 and 11).

It is a curious finding that, under the conditions of the experiment in Fig. 2, PC-II$_{INT}$ preincubated without dsRNA does not phosphorylate eIF-2 in the crude ribosome preparation when subsequently tested for kinase activity in the presence of high concentrations (20 μg/ml) of dsRNA and ATP (track 10).

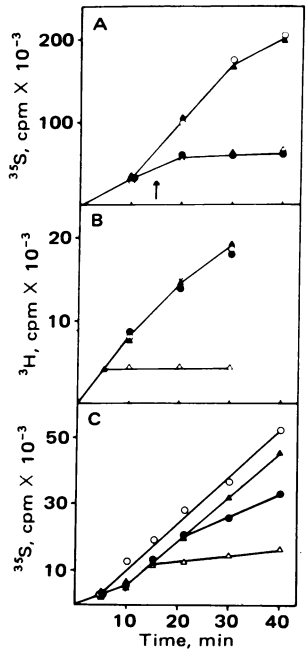

FIG. 4. Effect of pppA(2′p5′A)$_n$ on translation in reticulocyte lysate of (*A*) added Mengo virus RNA, (*B*) endogenous mRNA, and (*C*) added globin mRNA. (*A*) Reaction mixtures (22 μl) contained mRNA-dependent reticulocyte lysate, a mixture of [^{35}S]methionine and [^{35}S]cysteine (2.5 × 10^5 cpm/μl of reaction mixture), and 20 μg of Mengo virus RNA per ml (prepared as in ref. 28). Further additions were 60 nM pppA(2′p5′A)$_n$ [prepared by the poly(I)·poly(C) column procedure] treated with micrococcal nuclease to degrade dsRNA (●,△), an equal volume of buffer similarly treated with micrococcal nuclease (▲), and an equal volume of water (○). Incubation was at 30°C; after 15 min (arrow) 2.5 μg of highly purified eIF-2 from rabbit reticulocyte lysate was added to one of the reaction mixtures (△). Aliquots of 4 μl were taken at the times indicated for assay of protein synthesis. The nuclease activator was treated with 1500 units of micrococcal nuclease per ml (Worthington) in 1 mM $CaCl_2$ at pH 7.5 and 30°C for 30 min. Digestion was terminated with excess EGTA [ethylene glycol bis(2-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid], which complexes Ca^{2+} ions and thereby blocks micrococcal nuclease activity. (*B*) Standard reticulocyte lysate protein-synthesizing system (endogenous mRNA and [^{3}H]leucine) was used. Additions were 60 nM pppA(2′p5′A)$_n$ treated with micrococcal nuclease (●), an equal volume of buffer similarly treated with micrococcal nuclease (▲), an equal volume of water (○), and 50 ng of dsRNA per ml (△). Incubation and processing were as in *A*. (*C*) Reaction mixtures (22 μl) contained mRNA-dependent reticulocyte lysate, [^{35}S]methionine and [^{35}S]-cysteine as in *A*, and 20 μg of rabbit globin mRNA per ml. Further additions were 60 nM pppA(2′p5′A)$_n$ treated with micrococcal nuclease (●), an equal volume of buffer similarly treated with micrococcal nuclease (▲), an equal volume of water (○), and 50 ng of dsRNA per ml (△). Incubation and processing were as in *A*.

Preliminary experiments indicate that this is because the crude ribosome preparation prevents activation of PC-II$_{INT}$ by dsRNA under these conditions (data not shown).

Unless otherwise indicated in these experiments (just as in those in Fig. 1), the effect of carry-over of dsRNA from the preincubations was circumvented by adding dsRNA at high concentrations (20 μg/ml) to the incubation mixtures in which protein phosphorylation was tested. The fact that the endogenous dsRNA-dependent protein kinases in crude ribosomes are not activated at such high concentrations of dsRNA (compare tracks 6 and 7) agrees with earlier reports (15, 26).

PC-II$_{INT}$ Does Not Generate pppA(2′p5′A)$_n$ Even in Presence of dsRNA, and pppA(2′p5′A)$_n$ Does Not Substitute for dsRNA in Activating the Protein Kinase System in PC-II$_{INT}$. dsRNA and ATP added to an extract from interferon-treated cells generate a heat-stable product [pppA(2′p5′A)$_n$] (11) that

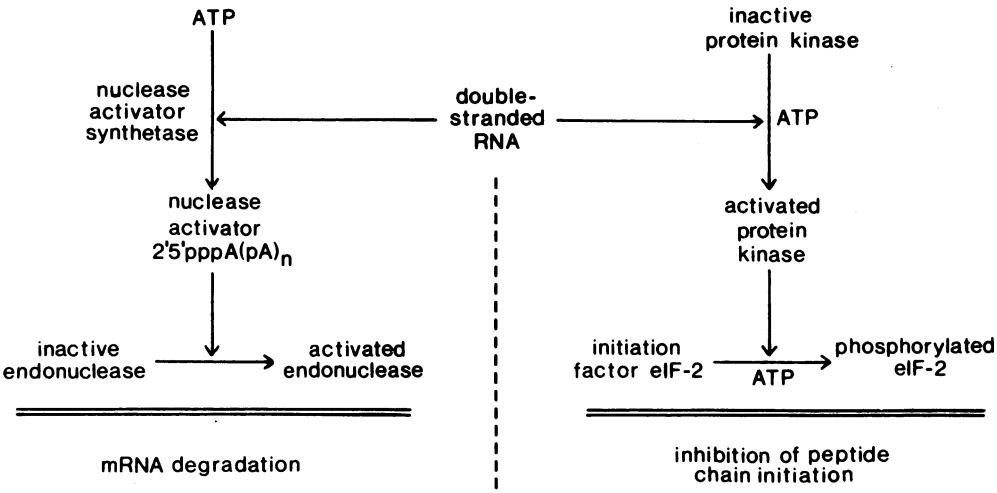

FIG. 5. Two pathways for inhibition of protein synthesis by dsRNA.

acts as the nuclease activator (8, 9) in the activation of a latent endonuclease by dsRNA and ATP (5–7). To assay the latent endonuclease (Fig. 3 *left*) we incubated R17 bacteriophage [^{32}P]RNA with the latent endonuclease preparation without (track 1) or with pppA(2'p5'A)$_n$ (track 2). Thereafter we determined the effect of the incubation on the labeled RNA by NaDodSO$_4$ gel electrophoresis and radioautography. The activation of the latent endonuclease by pppA(2'p5'A)$_n$ is revealed in the pattern of cleaved RNA in track 2.

We wondered if pppA(2'p5'A)$_n$ might also be a mediator in the activation of the protein kinase by dsRNA and ATP. To test if PC-II$_{INT}$ generates pppA(2'p5'A)$_n$ we preincubated PC-II$_{INT}$ with ATP and dsRNA and then heated the reaction mixture and added it to the partially purified latent endonuclease. The data in Fig. 3 *left* (tracks 3 and 4) revealed no acceleration of R17 bacteriophage RNA cleavage in this reaction mixture. Using this type of assay we failed to detect pppA(2'p5'A)$_n$ synthesis by PC-II$_{INT}$ not only under conditions of Fig. 3 *left*, which are optimal for pppA(2'p5'A)$_n$ synthesis by an enzyme preparation (DE-1) (8) purified from EAT cells for synthesizing pppA(2'-p5'A)$_n$, but also under those optimal for protein phosphorylation by PC-II$_{INT}$ (19). Moreover, pppA(2'p5'A)$_n$ used at a concentration 2 or 10 times higher than needed for endonuclease activation does not substitute for dsRNA in activating the dsRNA-dependent protein kinase(s) in crude ribosomes (Fig. 2 *right*, track 19) or in PC-II$_{INT}$ (not shown). These results seem to indicate that pppA(2'p5'A)$_n$ is neither an activator nor a product of PC-II$_{INT}$.

Activation of a Latent Endonuclease in Reticulocyte Lysate by pppA(2'p5'A)$_n$ or by dsRNA at High Concentrations. Hovanessian and Kerr (13) reported that, in a reticulocyte lysate supplemented with dsRNA and ATP, pppA(2'p5'A)$_n$ is formed and inhibits protein synthesis. The findings that dsRNA (5) and ATP (6) or pppA(2'p5'A)$_n$ activate a latent endonuclease in an extract of EAT cells prompted us to test the effect of these agents on RNA degradation in a reticulocyte lysate. The data in Fig. 3 *right* reveal that pppA(2'p5'A)$_n$, prepared by the poly(I)·poly(C) column procedure with an extract from interferon-treated EAT cells, accelerates bacteriophage R17 RNA cleavage in a reticulocyte lysate (track 6). However, the poly(I)·poly(C) column procedure with an extract from control EAT cells does not result in the production of (sufficient) pppA(2'p5'A)$_n$ to affect the cleavage in the lysate (track 7). Addition of dsRNA at high concentration (20 μg/ml) accelerates RNA cleavage in the lysate (track 8); however, dsRNA at low concentration (50 ng/ml) has no detectable effect (track 9). Omission of heme does not result in acceleration of RNA cleavage (track 11).

The acceleration by pppA(2'p5'A)$_n$ of RNA cleavage in the reticulocyte lysate is analogous to the effect of this compound on extracts from interferon-treated EAT cells (8). This acceleration of RNA cleavage agrees with the recent findings of Clemens and Williams (27), who reported an acceleration of mRNA inactivation by pppA(2'p5'A)$_n$ in reticulocyte lysates and suggested that it activates a nuclease.

The fact that RNA cleavage in reticulocyte lysates is accelerated by dsRNA only at high concentrations is in remarkable contrast with eIF-2 phosphorylation, which is activated by dsRNA only at low concentration. We now know that this reflects a difference in the mechanisms of activation of the protein kinase and endonuclease.

Translation of Some mRNAs Is More Susceptible to pppA(2'p5'A)$_n$ than That of Other mRNAs. We tested the effect of pppA(2'p5'A)$_n$ on the translation of Mengo virus RNA in an mRNA-dependent reticulocyte lysate protein-synthesizing system. The curves in Fig. 4*A* indicate that pppA(2'p5'A)$_n$ completely inhibits the translation of Mengo virus RNA in this system in about 20 min. Moreover, as expected for an inhibition due to mRNA cleavage, this is not overcome by added eIF-2. This emphasizes the difference in mode of action of pppA(2'-p5'A)$_n$ and activated PC-II$_{INT}$.

The translation of endogenous mRNA in a (not mRNA-dependent) reticulocyte lysate is not detectably affected by pppA(2'p5'A)$_n$, at least during the time span of a 30-min experiment (Fig. 4*B*). It is stopped, however, within less than 10 min by dsRNA added at a low concentration. The translation of exogenous globin mRNA in the mRNA-dependent reticulocyte lysate protein-synthesizing system is inhibited by pppA(2'p5'A)$_n$ (Fig. 4*C*), but to a much smaller extent than the translation of Mengo virus RNA.

The difference in susceptibility to pppA(2'p5'A)$_n$ between Mengo virus RNA translation and exogenous globin mRNA translation is probably due to a difference in the rate of cleavage of the two mRNAs by the activated endonuclease. Indeed, globin mRNA is cleaved only very slowly by the activated endonuclease in an extract from interferon-treated EAT cells (7). The difference in susceptibility to pppA(2'p5'A)$_n$ of the translation of exogenous globin mRNA in the mRNA-dependent lysate and endogenous protein synthesis, most of which consists of globin translation, might have several causes. The somewhat slower rate of protein synthesis with the mRNA-dependent system as compared with the endogenous system (21) may lead to less protection of mRNA from the endonuclease by ribosomes in polysomes. Also, the fact that at the beginning of the experiment the mRNA-dependent system con-

tains naked mRNA and the endogenous system contains mRNA in polysomes may be important in this respect. It remains to be seen whether the difference in susceptibility of mRNAs to the pppA(2′p5′A)$_n$-activated endonuclease either because of the size of the mRNAs (Mengo virus RNA is much longer than globin mRNA) or because of the sequence and secondary structure contributes to the selectivity of interferon action.

DISCUSSION

There are two separate pathways for the impairment of protein synthesis by dsRNA. Lewis *et al.* (29) recently examined the effects of dsRNA on unfractionated extracts of interferon-treated L cells and concluded that dsRNA impairs protein synthesis at the level of initiation and by mRNA degradation. The results presented here were obtained in experiments with fractionated enzyme systems. They indicate the existence of at least two separate pathways for the inhibition of protein synthesis by dsRNA (Fig. 5). Both require ATP. The pathway resulting in the inhibition of peptide chain initiation involves the action of a protein kinase system. If activated by dsRNA and ATP, this system phosphorylates and thereby (30, 31) inactivates the peptide chain initiation factor eIF-2. The pathway resulting in mRNA degradation involves the action of at least two enzymes. The first one, if activated by dsRNA, synthesizes the oligonucleotide pppA(2′p5′A)$_n$, which activates the second enzyme, an inactive endonuclease.

In EAT cells (from which these enzymes were partially purified), interferon treatment increases the level of the enzymes leading to mRNA degradation and to eIF-2 phosphorylation severalfold (unpublished data). The presence of both pathways in reticulocytes not treated with interferon indicates that the above systems for translational control may not only be components of the interferon system.

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