Four Different Forms of Interferon-induced 2',5'-Oligo(A) Synthetase Identified by Immunoblotting in Human Cells*

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Antibodies against synthetic peptides derived from the cDNA sequence of interferon-induced 2',5'-oligo(A) synthetase, and which immunoprecipitate the native enzyme activity, were found to detect multiple enzyme forms in denaturing electrophoretic immunoblots. In some human cell lines, four different interferon-induced proteins of 40, 46, 67, and 100 kDa were found to react with the same peptide antibodies. Each isolated form was shown to have 2',5'-oligo(A) synthetase activity, but the dependence on double-stranded RNA was markedly different for activation of the individual enzymes. The four enzyme forms also differ in their intracellular localization, on microsomes (100 kDa), in nuclei (67, 46, 40 kDa), and on membrane structures (67 kDa). Plasma membranes from interferon-treated Daudi lymphoblastoid cells are highly enriched in the 67-kDa 2',5'-oligo(A) synthetase form. The 2',5'-oligo(A) synthetase activity induced by interferons in human cells appears, therefore, as a complex multienzyme system.

A 2',5'-oligo(A) synthetase activity which polymerizes ATP into ppp(A2'p)nA oligomers is induced 10-100-fold in cells treated by all known interferon (IFN) species (for reviews see Refs. 1-3). At present, the only known function of these 2',5'-oligoadenylates is to activate a latent ribonuclease (1'-thetase activity shows cell cycle-dependent variations, being cell cycle-dependent and their involvement in IFN action or other cellular events.

Attempts to purify the 2',5'-oligo(A) synthetase activity from human and mouse cells have identified large 100-kDa (16, 17) and small 30-40-kDa (18) enzyme forms, which often coexist in the same cells (4, 19-21) and appear in cytoplasm and in nuclei (4, 9, 14, 20). The molecular cloning of human 2',5'-oligo(A) synthetase cDNA (22) revealed further heterogeneity in the enzyme forms. Thus, we isolated two cDNAs, one for a 1.6-kb (E16) mRNA and the other for a 1.8-kb (E18) mRNA, originating by differential processing from the same gene (23) and predicting two proteins of 40 and 100 kDa. Those sequences differ in the carboxyl-terminal region (24). To verify the existence of these multiple 2',5'-oligo(A) synthetase forms in human cells, we have now used the cDNA sequence information to derive synthetic peptide antigens and elicit antibodies against the native enzyme. Electrophoretic immunoblotting demonstrates that human cells can contain four different forms of IFN-induced 2',5'-oligo(A) synthetase corresponding to proteins of 40, 46, 67, and 100 kDa localized in various compartments of the cells. All four isolated forms synthesize 2',5'-oligo(A), but differ in their requirement for double-stranded RNA activation. The immunoblot procedure also shows cell-specific expression of the various 2',5'-oligo(A) synthetase forms, suggesting differences in their regulation and their involvement in IFN action or other cellular events.

MATERIALS AND METHODS

cDNA Transcription-Translation—EcoRI inserts of E16 cDNA clone 9-21 (24) and E18 cDNA clone 18-13-E, isolated by plaque hybridization from a λgt11 (25) cDNA library from IFN-β-treated Daudi cells, were subcloned in pGEM-1 (Promega-Biotec). After T7 RNA polymerase transcription of 1 µg of plasmid BamHI cut, 0.05 µg of sense RNA (alone or mixed with 2- and 10-fold excess antisense RNA) were translated in 25-µl reactions containing 6 µl of reticulocyte lysate pretreated with micrococcal nuclease and 6 µCi of [35S]methionine (>800 mCi/mmol, Amersham Corp.) (24). 2',5'-oligo(A) synthetase activity was tested in 2-µl aliquots as below.

Antibodies to 2',5'-Oligo(A) Synthetase—Two peptides from the E16 and E18 sequences (peptide A: Glu-Lys-Tyr-Leu-Arg-Gln-Leu-Thr-Lys-Pro-Arg-Pro-Val-Ile-Leu-Asp-Pro-Ala-Asp and peptide C: Arg-Pro-Pro-Ala-Ser-Ser-Leu-Pro-Phe-Ile-Pro-Ala-Leu-His-Glu-Ala), synthesized by the solid-phase method of Barany and Merrifield (26) and purified on Sephadex G-25 in 2 M acetic acid, were coupled to Keyhole Limpet hemocyanin (Behring Diagnostics). Peptide B was coupled by ethylenediamine carbodiimide (27) and peptide C through its NH2 terminus esterified with p-aminophenylacetic acid (28). Rabbits were injected subcutaneously with 1 mg of conjugate (0.2 mg of peptide) in complete Freund's adjuvant, boosted twice at 2-week intervals with 0.5 mg of conjugate in incomplete adjuvant, and subsequently with 0.1 mg of carrier-free peptide in incomplete adjuvant until maximal antibody response. Antibody titers of rabbit sera were measured by enzyme-linked immunosorbent assays (29) using the carrier-free peptides.

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grown in suspension to 1.5×10^6 cells/ml as before (22, 23). Cultures were treated for 16-24 h with 500 units/ml human rIFN-β, produced in Chinese hamster ovary cells and purified to homogeneity on monoclonal antibodies (30). For detergent extracts, cells were washed twice with phosphate-buffered saline (all steps at 4°C) and lysed in Buffer B containing 0.5 M NaCl, 0.1% Nonidet P-40, and 10% glycerol. Lysates were centrifuged for 2 h at 15,000 × g for 30 min at 4°C. Fractions of 0.2 ml were collected and 2',5'-oligo(A) synthetase activity tested on 0.5-2 μl aliquots while 20 μl were analyzed on electrophoretic immunoblots.

Nuclear and plasma membranes were prepared without detergent lysis. Daudi cells subjected to 90% glycerol loading (32) were homogenized in hypotonic Buffer B (10 mM Hepes buffer, pH 7.5, 1 mM MgCl₂, 1 mM CaCl₂, 1 mM phenylmethylsulfonyl fluoride). The 700 × g pellet was Deuc homogenized again in Buffer B, and the second 700 × g pellet was a nucleo-free intact cells or large membrane fragments visible in the microscope. From the 700 × g supernatant, a 15,000 × g pellet was mixed with Buffer B containing 0.5 M sucrose, loaded over a 5-Ml cushion of 0.5 M sucrose, 0.5 M Ficoll (Pharmacia) in Buffer B, and centrifuged in the Sorvall HB4 swinging rotor at 30,000 × g for 30 min to purify plasma membranes floating at the interphase (33). PS11 diploid fibroblasts, washed with 20 mM Hepes buffer, pH 7.5, 150 mM NaCl, were swollen in Buffer A with 0.1 mM EDTA, 1 mM MgCl₂, 10 mM potassium acetate, 1 mM dithiothreitol, 1.5 mM ATP, 4 μCi of [α-32P]ATP, 50 μM poly(rI)(rC) (from Pharmacia P-L Biochemicals) for 2 h at 30°C. After boiling for 5 min and microcentrifugation, bacterial alkaline phosphatase was added to 25 units/ml for 2 h at 37°C. From 2 to 7 μl were electrophoresed on Whatman 3MM at 3000 V in pyridine/acet acid, pH 3.5. After autoradiography, (the A2′)a) oligomers spots were cut out and counted (18).

For immunoprecipitation, 5-10 μl of anti-2',5'-oligo(A) synthetase rabbit serum was adsorbed on 3 mg of Protein A-Sepharose (Phar macia) in phosphate-buffered saline with 3% bovine serum albumin for 30 min at room temperature and then washed with phosphate-buffered saline-1% bovine serum albumin. About 2 μg of S15 proteins in 20 μl of buffer A were added to the pelleted Protein A-Sepharose, washed, and mixed for 2 h at 4°C. The suspension diluted 5-fold in buffer A was spun, and the pellet was washed 3 times with 0.5 μl of buffer A and then suspended in 25 μl of the enzyme reaction mixture. Activity was measured also in the nonbound supernatant.

Electrophoretic Transfer Immunoblot-Proteins from cellular fractions (30 μg) were heated and electrophoresed in sodium dodecyl sulfate on 7.5 or 10% polyacrylamide gels with [-C]methylated proteins (Amersham Corp.) as molecular weight markers. Electrophoretic transfer (34) onto nitrocellulose paper (Schleicher and Schuell BA85) was done in 25 mM Tris base, 192 mM glycine, and 20% methanol. The blots were incubated in 10 ml of 0.9 M NaCl, 0.01 M Tris-HCl, pH 7.5, 10% (v/v) of a 1%-fat milk solution, 15% (v/v) heat-inactivated fetal calf serum, and 0.05% Tween 20, overnight at 4°C, followed by 30 min at 37°C, and then incubated with the addition of peptide antibodies in the form of antiserum IgG (0.1 mg/ml) or preimmune IgG (0.1 mg/ml) for 1 h at 37°C. Blots were washed 5 times for 10 min in 4% fetal calf serum, incubated in the above preincubation mixture with 10^6 cpm/ml 32P-Protein A (Amersham Corp., 30 μCi/ml) for 1 h at 37°C, washed, and subjected to autoradiography on Agfa-Curix films.

RESULTS

Peptide Antibodies Recognizing Native 2',5'-Oligo(A) Synthetase—Antibodies to human 2',5'-oligo(A) synthetase were obtained in rabbits with synthetic peptides derived from the sequence of the cloned cDNAs. The two proteins predicted by the E16 and E18 cDNAs (24) have amino acids 1-346 in common followed in E16 by 18 hydrophobic carboxyl-terminal residues and in E18 by 54 carboxyl-terminal acidic residues. Antiserum B was raised against a peptide comprising amino acids 284-303 in the part common to E16 and E18. Antiserum C was raised against the carboxyl-terminal residues 348-364 specific to E16. We first verified that the peptide antibodies recognize and bind 2',5'-oligo(A) synthetase activity. Immunoglobulins from antiserum B and C, or from preimmune rabbit serum, were immobilized on Protein A-Sepharose beads and mixed with extracts of IFN-treated Wish cells. The proteins adsorbed to the beads were assayed for 2',5'-oligo(A) synthetase activity after adding poly(rI)(rC) and [α-32P]ATP. The B and C antibodies adsorbed IFN-induced 2',5'-oligo(A) synthetase activity, respectively, 7- and 25-fold more than preimmune serum-coated beads (Table I). The high efficiency of C antibodies is in line with our previous observation (23) that Wish cells express predominantly the 1.6-kb mRNA (E16). In contrast, with Daudi cells, which express only the 1.8-kb mRNA (E18) (23), the B antibodies immunoprecipitated 2',5'-oligo(A) synthetase activity but C antibodies did not, demonstrating their specificity and the absence of E16 product in Daudi cells. The more general B antibodies were, therefore, chosen to analyze the 2',5'-oligo(A) synthetase proteins in human cell extracts.

Immunoblots of 2',5'-Oligo(A) Synthetase in Human Cells—To visualize the enzyme, we looked for IFN-β-induced proteins recognized by B antibodies on electrophoretic blots of 15,000 × g supernatants from human cells which had been lysed by Nonidet P-40, conditions which maximally extract the enzymatic activity (18). Human cell lines Daudi, Wish, and SV80 were compared because of their differing expression patterns for the 1.6- and 1.8-kb mRNAs (23). The products expected from these mRNAs are two enzymatically active proteins migrating, respectively, at 40 and 46 kDa, as demonstrated by the in vitro transcription-translation of E16 and E18 cDNAs (Fig. 1). The immunoblots confirmed the presence in human cells of IFN-induced proteins corresponding to the predicted 38-40 and 43-46-kDa sizes (Fig. 2). Daudi cells (lane 1) strongly expressed the 46-kDa E18 protein but not the 40-kDa E16 protein in line with the absence of 1.6-kb RNA in these cells (23). Conversely, Wish cells had no 46- kDa 2',5'-oligo(A) synthetase but expressed the 40-kDa protein (Fig. 2, lane 2). SV80 cells, which make both 1.6- and 1.8-kb mRNAs (23), showed both 40- and 46-kDa IFN-induced proteins recognized by B antibodies (Fig. 2, lane 3).

In the same immunoblots, however, the B antibodies strongly detected also two large proteins of 67-69 kDa and of 100 kDa, both induced by IFN (Fig. 2). Like the small 2',5'-oligo(A) synthetase forms, the 100-kDa protein showed cell-specific expression and was very low in Daudi cells (lane 1).

**Table I**

<table>
<thead>
<tr>
<th>Wish cells</th>
<th>Daudi cells</th>
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<tr>
<td>anti-B</td>
<td>anti-C</td>
</tr>
<tr>
<td>cpm</td>
<td>cpm</td>
</tr>
<tr>
<td>IFN-treated</td>
<td>12,425</td>
</tr>
<tr>
<td>Untreated</td>
<td>4,345</td>
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Normal serum background subtracted: 2,130 for IFN-treated, 1,430 for untreated. The immunoprecipitation procedure is described under "Materials and Methods."
IFN Induces Four 2',5'-Oligo(A) Synthetases in Human Cells

Fig. 1. Transcription-translation of 2',5'-oligo(A) synthetase E18 and E16 cDNAs. In vitro transcribed sense and antisense E18 and E16 RNAs (see “Materials and Methods”) were translated in reticulocyte lysates at indicated concentrations and the [38S]methionine-labeled products analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. M, molecular weight markers. Lanes 1 and 2, 0.05 μg of E16 RNA; lanes 3 and 4, E18 RNA mixed with 0.5 or 0.1 μg of antisense E18 RNA; lane 5, no mRNA; lanes 6 and 7, 0.05 μg of E16 RNA; lanes 8 and 9, E16 RNA mixed with 0.5 or 0.1 μg of antisense E16 RNA. 2',5'-Oligo(A) synthetase activity was: without pg of E16 RNA; 330 cpm; for E18 RNA, 2920 cpm; and for E16 RNA, 4430 cpm in reticulocyte lysates at indicated concentrations and the [35S]methionine-labeled products analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. M, molecular weight markers: myosin (200,000), phosphorylase b (92,500), bovine serum albumin (68,000), ovalbumin (46,000), carbonic anhydrase (30,000).

Fig. 2. Immunoblots of 2',5'-oligo(A) synthetase in subcellular fractions. Extracts of human cell lines treated by IFN-β for 18 h (+) or untreated (−) were prepared with 0.5% Nonidet P-40 in Buffer A (lanes 1–6) or without detergent in Buffer B (lanes 7–9). Either 30 μg of protein (lanes 1–3) or 3 μg of protein (lanes 4–9) were electrophoresed on 10% (lanes 1 and 2) or 7.5% (lanes 3–10) polyacrylamide gels in sodium dodecyl sulfate, electroblotted onto nitrocellulose, reacted successively with B antibodies and 125I-Protein A, and autoradiographed for 24 h. Lanes 1–3, 15,000 × g supernatant from Daudi, Wish, and SV80 cells. Lanes 4–6, 100,000 × g supernatant, 0.5 M KCl ribosomal wash fluid, and KC1-washed ribosomes of SV80 cells. Lanes 7–9, plasma membranes, nuclei-rich fraction, and 15,000 × g supernatant (without detergent) from Daudi cells. Lane 10, purified nuclei from FS11 cells. M, molecular weight markers: myosin (200,000), phosphorylase b (92,500), bovine serum albumin (68,000), ovalbumin (46,000), carbonic anhydrase (30,000).

Fig. 3. Isolation of 2',5'-oligo(A) synthetase forms from SV80 cells. Upper panel, immunoblots with B antibodies of fraction from Nonidet P-40 lysed SV80 cells. Analysis was done on 7.5% polyacrylamide gels in sodium dodecyl sulfate. M, molecular weight markers. Lanes 1 and 2, 30 μg of protein from 15,000 × g supernatant (as in Fig. 2, lane 3) of IFN-β-treated (2) or untreated (1) cells. Lane 3, ribosomal wash fluid from IFN-β-treated cells (as in Fig. 2, lane 5, +) applied to DEAE-cellulose in Buffer A and eluted with 0.15 M KCl. Note strong 100-kDa and weak 46-kDa bands. Lanes a, the preparation seen in lane 3 (20 μg) was sedimented on glycerol gradient and fractions 13–23 were analyzed by immunoblot. Lower panel, correlation between 2',5'-oligo(A) synthetase activity and proteins in glycerol gradient fractions. Panel a, glycerol gradient shown in lanes a above. From each 0.2-ml fraction collected, 2 μl were assayed for 2',5'-oligo(A) synthesis in the presence of 50 μg of poly(rI)(rC) (closed circles). In parallel, the intensity of the 100-kDa band was recorded by scanning the autoradiography of lanes a above and expressed in percent of maximal intensity (open triangles). Panel b, glycerol gradient shown in lanes above. Enzymatic activity was assayed on 0.5 μl of each gradient fraction, and the intensity of the 40-kDa band was recorded.

as compared to Wish and SV80 (lanes 2 and 3). These proteins were not seen in blots with nonimmune rabbit sera (not shown). The next experiments were undertaken to verify that the additional proteins recognized by B antibodies are indeed large 2',5'-oligo(A) synthetase forms.

Isolation of Four Active 2',5'-Oligo(A) Synthetases—Human cell extracts were fractionated to isolate from each other the different IFN-induced proteins seen on the immunoblots. Centrifugation of the Nonidet P-40 crude extract from SV80 cells showed that the 100-kDa protein was absent from the 100,000 × g supernatant (S100; Fig. 2, lane 4) and concentrated in the microsomal pellet from which it can be partially released by 0.5 M KCl (Fig. 2, lanes 5 and 6). The 2',5'-oligo(A) synthetase activity in this KCl-ribosomal wash fluid was retained on DEAE-cellulose (21) and eluted with 0.15 M KCl. An immunoblot showed that the DEAE eluate contains the 100-kDa protein with small amounts of the 46-kDa species (Fig. 3, top, lane 3). These could be separated by sedimentation on a glycerol gradient which shows (Fig. 3, lanes a and panel a) the cosedimentation of the 100-kDa protein measured by
immunoblots (open triangles), with the bulk of 2',5'-oligo(A) synthetase activity, whereas the 46-kDa protein was detected only in the lighter enzyme peak. Thus, the 100-kDa protein is an active enzyme.

Upon chromatography of the S100 on DEAE-cellulose, much of the 2',5'-oligo(A) synthetase activity is unbound in low salt (18, 21) but is retained on Cm-cellulose and eluted at 0.5 M KCl. This fraction contains only the 40-kDa protein (Fig. 3, top, lane 4) and gave a single peak of 2',5'-oligo(A) synthetase activity in the glycerol gradient, coinciding with the sedimentation pattern of the 40-kDa protein (Fig. 3, lanes b and panel b). The 67- and 46-kDa proteins present in SV80 cell S100 (Fig. 2, lane 4) were both adsorbed to DEAE-cellulose, possibly the acidic carboxyl terminus of the 46-kDa protein (24) retaining it on the anion exchanger. The 67-kDa protein was isolated from detergent-extracted Daudi cells S15, where it is most abundant (Fig. 4, left) by chromatography on DEAE-cellulose (Fig. 4, right). 2',5'-Oligo(A) synthetase activity was eluted by 0.1 M KCl, but a significant proportion of the enzyme eluted only with higher salt concentrations. Immunoblots of these latter 0.5 M KCl fractions with peak 2',5'-oligo(A) synthetase activity showed the presence of the 67-kDa protein free of the other forms (Fig. 4, left, lanes 38 and 40). The hitherto undescribed 67-kDa 2',5'-oligo(A) synthetase form has, therefore, enzymatic activity.

The 2',5'-Oligo(A) Synthetases Differ in Their dsRNA Requirements—The enzymatic activities of the peak fractions from the glycerol gradients in Fig. 3, corresponding, respectively, to the 100-kDa heavy peak, panel a) and 40-kDa 2',5'-oligo(A) synthetase (panel b) were tested in solution with various concentrations of poly(rI)-(rC). Fig. 5 shows that the 100-kDa form was maximally active at dsRNA concentrations which are 1000 times smaller than those needed for optimal activation of the 40-kDa E16 protein. The 100-kDa 2',5'-oligo(A) synthetase appears to have significant activity without added dsRNA, and high concentrations of poly(rI)(rC) were even slightly inhibitory for the 100-kDa enzyme form. In contrast, the 40-kDa 2',5'-oligo(A) synthetase was inactive without dsRNA, and activity increased from 10 to 100 μg/ml poly(rI)(rC). The 46-kDa enzyme was also dsRNA dependent and stimulated by high concentrations of poly(rI)(rC).

The enzymatic activity of the small 40-kDa (2',5') enzyme also differed from that of the 100-kDa synthetase by its higher ratio of dimer to longer oligo(A) nucleotide; under optimal conditions of activity, the ratio of dimers to trimers was 10 for the 40-kDa and 3 for the 100-kDa enzymes.

Distribution of the Different Enzymes in Subcellular Fractions—Fractionation of Nonidet P-40 extracts of SV80 cells showed that the different IFN-induced 2',5'-oligo(A) synthetase proteins were not evenly distributed in the subcellular fractions. Most of the 100-kDa protein was found associated with microsomes (Fig. 2, lane 5) and poorly represented in other cell fractions. The cytosol S100 contained much more 40-kDa than 46-kDa protein (Fig. 2, lane 4) while microsomes had much more 46-kDa than 40-kDa 2',5'-oligo(A) synthetase (lane 6). The 67-kDa protein was present in all fractions obtained from detergent-lysed cells.

Since lysis of cells with detergents may release 2',5'-oligo(A) synthetase from cellular structures, we investigated the localization of the protein in cells disrupted without detergent. Under these conditions, the concentration of 2',5'-oligo(A) synthetase proteins released in the 15,000 × g supernatant of Daudi cells was indeed low (Fig. 2, lane 9) in comparison to the nuclei-rich 700 × g pellet analyzed at the same protein concentration (lane 8). This nuclear fraction contained both 67- and 46-kDa forms. Unexpectedly, we found that 2',5'-oligo(A) synthetase was also abundant in the membrane-rich 15,000 × g pellet derived from the nuclei-free 700 × g supernatant. Plasma membranes, purified from this pellet by flotation on 0.5 M sucrose, 0.5 M Ficoll, showed a high 2',5'-oligo(A) synthetase enzymatic specific activity, and immunoblots revealed that the plasma membranes contain exclusively the 67-kDa protein (Fig. 2, lane 7). Without detergent, Daudi cells have, therefore, little 2',5'-oligo(A) synthetase in the cytosoluble protein fraction, most of it being bound to cellular structures including the plasma membrane. Immunofluorescence studies confirmed that B antibodies stain the membranes of live human lymphoid cells (not shown).

FS1 fibroblasts which, like SV80, display all 4 forms of 2',5'-oligo(A) synthetase were also fractionated without detergent lysis. The 2',5'-oligo(A) synthetase proteins were then enriched in the particulate fractions as compared to the cytosoluble fractions. While the 100- and 46-kDa forms were...
seen clearly only in the microsomal pellet, the 67- and 40-kDa forms were more abundant in the nuclear-rich 3,000 × g pellet and membrane-rich 15,000 × g pellet (not shown). To investigate which forms of the enzyme are present in nuclei of FS11 fibroblasts, the 3,000 × g pellet was treated by Nonidet P-40, and the nuclei were purified by centrifugation through a 25% glycerol cushion. Immunoblots showed that the purified fibroblast nuclei contain both the IFN-induced 67- and 40-kDa 2',5'-oligo(A) synthetase forms (Fig. 2, lane 10).

**DISCUSSION**

Heterogeneity in IFN-induced 2',5'-oligo(A) synthetase activity was first noticed by gel filtration and sedimentation analyses of extracts from human (19) and mouse (4) cells, in which large (80–100 kDa) and small (30–40 kDa) enzyme forms were detected. Purified preparations of 2',5'-oligo(A) synthetase from HeLa cells were shown to contain a 105-kDa polypeptide (16, 17) while an enzyme purified from Namalva cells appeared to correspond to the smaller form (18). The present work shows that the 2',5'-oligo(A) synthetase actually represents a system of at least four IFN-inducible enzymes differing in their molecular weights, biochemical properties, and affinity for cellular structures. We have previously determined the sequence of two of these 2',5'-oligo(A) synthetase proteins (40 and 46 kDa, respectively) by cloning their respective cDNAs (24). Both are active enzymes as shown by cDNA transcription-translation and expression in Escherichia coli (35).² Here we used the sequence information on these proteins to obtain peptide antibodies and demonstrate that both the 40- and 46-kDa proteins are indeed made in human cells in response to IFN. The same peptide antibodies as the E16 and E18 cDNA products, we suggest, specifically. Since they are recognized by the same peptide B "antibodies" do not precipitate 2',5'-oligo(A) synthetase activity from Daudi cells, but efficiently do so in extracts of Wish cells. This also indicates that the 67-kDa protein of Daudi does not have the same carboxyl terminus as the 40-kDa protein. The 100-kDa 2',5'-oligo(A) synthetase is expressed only at very low levels in Daudi cells, as compared to the other human cell lines studied here. The fact that three of the 2',5'-oligo(A) synthetase forms display cell type-specific expression suggests that they are subject to different developmental regulations.

The mode of synthesis of the two large 2',5'-oligo(A) synthetase forms in human cells remains to be determined precisely. Since they are recognized by the same peptide B antibodies as the E16 and E18 CDNA products, we suggest that all four human 2',5'-oligo(A) synthetase forms share a common epitope and hence that their mRNAs could be sequence related. Large 2',5'-oligo(A) synthetase-specific RNAs of 4, 3.6, and 2.7 kb were detected in Northern blots with E16 and E18 cDNAs (23). Although some of these RNAs may be partially processed precursors of E18 (23), the kinetics of their accumulation in SV80 and FS11 cells (22) would argue against their being all precursors. In mouse cells, a 5.5-kb RNA was found which produces 2',5'-oligo(A) synthetase sedimenting at 100 kDa, in addition to a 1.5-kb RNA producing a 30–40-kDa enzyme (4). A large and small pig 2',5'-oligo(A) synthetase also shares antigenic determinants (20). We favor, therefore, the possibility that both 2',5'-oligo(A) synthetase is encoded by a different mRNA. However, for the 67-kDa protein, the absence of large transcripts in Daudi cells (23) contrasts with the high amounts of this protein, and it remains possible that the 67-kDa protein is formed by post-translational modification of the 46-kDa enzyme. Cloning of active large 2',5'-oligo(A) synthetase mRNAs from human cells should answer these questions.

The complexity of the 2',5'-oligo(A) synthetase system would be in line with the hypothesis that it is involved in several different functions of IFNs as cell growth inhibition, cell differentiation, and virus resistance (1–3). Induction of 2',5'-oligo(A) synthetase and activation of the 2',5'-oligo(A)-RNase pathway in IFN-treated cells most probably mediates some of IFN's antiviral effects (1–3, 43). However, variations in 2',5'-oligo(A) synthetase activity have also been observed in relation with cell growth and differentiation (11–15), often due to autocrine IFN/β secretion (3, 14). The present study suggests that these changes in 2',5'-oligo(A) synthetase activ-

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² Y. Mory and J. Chebath, unpublished data.
³ A. Kimchi, private communication.
ity in the cells may not always be due to the induction of the same protein. Interestingly, in synchronized mouse embryo cells, a large 4–5-kb 2′,5′-oligo(A) synthetase RNA accumulates in parallel to the rise in enzyme activity at the end of the S phase in addition to the small 1.7-kb RNA induced by exogenous IFN in G1 cells (44). Hence, it will be important to re-examine the correlations and discrepancies between 2′,5′-oligo(A) synthetase induction and IFN effects (43) by following each of the protein forms and RNA species individually.

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