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Different mRNAs Induced by Interferon in Cells from Inbred Mouse Strains A/J and A2G

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Treatment of cells from inbred mouse strains A/J and A2G with interferon resulted in the development of different antiviral states for influenza viruses. A2G mice-derived cells that carry the resistance gene *Mx* were efficiently protected by interferon against influenza viruses, whereas the interferon protection against the same viruses in wild-type A/J mice-derived cells was only marginal. The two cell types, however, were equally protected by interferon against vesicular stomatitis virus and other non-orthomyxoviruses. The interferon-induced mRNAs of mouse embryonic fibroblast cells that carried either homozygous wild-type alleles or homozygous *Mx* alleles were compared. The isolated polysome-bound mRNAs from A/J (+/+) and A2G (*Mx*/*Mx*) cells were translated in a cell-free translation system, and the translation products were analyzed after two-dimensional gel electrophoresis. New mRNAs coding for at least eight proteins with molecular weights (MW) ranging from 30,000 to 80,000 were found in interferon-treated cells but not in control cells. Differences in the interferon-induced mRNAs from A/J and A2G cells were also found. An mRNA coding for a 72,000-MW protein was found in interferon-treated A2G cells but not in interferon-treated A/J cells. Interferon-treated A/J cells, on the other hand, contained an mRNA coding for a 65,000-MW protein that was not found in interferon-treated A2G cells. The in vitro-synthesized 65,000-MW protein efficiently bound to GMP. Cytoplasmic extracts prepared from interferon-treated A/J cells also contained a GMP-binding 65,000-MW protein that was undetectable in similarly treated A2G cells.

Treatment of animal cells with homologous interferon (IFN) results in the establishment of cellular resistance to most viral infections. Although it is still largely unknown what cellular changes must occur, both RNA transcription and protein synthesis are necessary for the establishment of an antiviral state (10). IFN induces two proteins in mouse and human cells which have been characterized as a 2′5′ oligo (A) synthetase and a protein kinase, respectively (1a, 11, 12). In addition, several IFN-induced mRNAs and proteins of unknown function have been identified (2–5, 7).

A mouse gene, *Mx*, affects the IFN efficiency in inhibiting influenza virus replication (for a review, see reference 6). Cells derived from A2G mice, which carry the *Mx* gene, can be protected against influenza viruses by much smaller amounts of IFN than can cells from non-*Mx*-bearing A/J mice. This different IFN-mediated antiviral effect is observed with influenza viruses only. The degree of antiviral protection against other viruses is independent of the *Mx* gene.

We have compared the IFN-induced mRNAs and proteins in cells from A/J and A2G mice. In this report, we show that an mRNA coding for a 72,000-dalton protein is induced by IFN in A2G cells but not in A/J cells. In addition, a new mRNA coding for a 65,000-molecular weight (MW) protein is synthesized in A/J cells but not in A2G cells. The in vitro-synthesized 65,000-MW protein as well as its in vivo counterpart have strong GMP-binding activities.

MATERIALS AND METHODS

Mice. A2G mice homozygous for the dominant gene *Mx* were bred at the Institute for Immunology and Virology, University of Zurich, Zurich, Switzerland. A/J mice lacking *Mx* were obtained from Jackson Laboratorires, Bar Harbor, Maine. The appropriate matings were performed at the facilities of the University of Zurich.

Cells. Cells from 16-day-old mouse embryos were prepared as described elsewhere (H. Arnheiter and P. Staeheli, Arch. Virol., in press). Cells were cultured in Dulbecco modified minimal essential medium (MEM) containing 10% fetal calf serum. Cells between pas-

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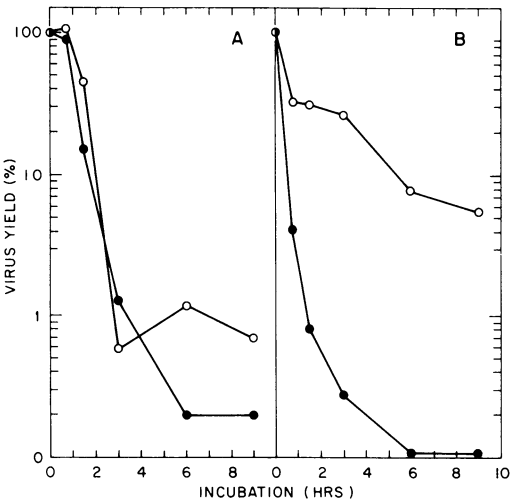

FIG. 1. Induction by IFN of the antiviral state against VSV and influenza virus in A/J and A2G cells. Individual 35-mm plates with confluent monolayers of A/J (○) and A2G (●) cells were treated with 100 reference U of IFN-β per ml for the time periods indicated and then infected with either VSV (A) or influenza A virus WSN (B). (A) VSV infection (multiplicity of infection, 3) was carried out at 34°C for 45 min in serum-free Dulbecco MEM containing 5 μg of actinomycin D per ml. Monolayers were washed and incubated for 16 h at 37°C in medium containing 2% fetal calf serum and 5 μg of actinomycin D per ml. Virus in the supernatants was assayed in an L929 cell plaque assay. (B) Influenza virus infection (multiplicity of infection, 1) was carried out at 34°C for 45 min in serum-free Dulbecco MEM. Monolayers were washed and incubated for 24 h at 37°C in medium containing 2% fetal calf serum. Virus in the supernatants was titrated in an MDBK cell plaque assay.

sages 3 and 6 were used for the experiments described below.

IFN. Partially purified mouse IFN-β (10^7 U/mg) was purchased from Lee Biomolecular, San Diego, Calif. Purified mouse IFN-α/β (5×10^8 U/mg) was purchased from Enzo Biochemicals, New York, N.Y. These IFN preparations were titrated with the L929 cell/VSV system, and the titers were found to be only about 20% of the manufacturer's values. The IFN concentrations given in the text represent our own titrated units.

Viruses. Influenza A virus WSN was obtained from R. M. Krug, Memorial Sloan-Kettering Cancer Center, New York, N.Y. Vesicular stomatitis virus (VSV) was the same as previously described (3).

mRNA isolation. mRNA isolation was the same as previously described (3).

In vitro translation. mRNAs were translated with the rabbit reticulocyte lysate in vitro protein synthesis system from Bethesda Research Laboratories, Bethesda, Md., in accordance with the manufacturer's protocol. The radioactive amino acid used was [^{35}S]methionine.

GMP-agarose-binding assay. GMP-agarose (Sigma Chemical Co., St. Louis, Mo.) was incubated with [^{35}S]methionine-labeled cytoplasmic extracts and thereafter extensively washed as described elsewhere (1b). The GMP-agarose-bound proteins were eluted with sodium dodecyl sulfate (SDS) gel sample buffer (8). In vitro translation products were diluted with two parts of 50 mM Tris-hydrochloride–5 mM $MgCl_2$–150 mM NaCl (pH 7.2) (1b), chromatographed on 3 volumes of GMP-agarose, and washed and eluted as described above.

Gel electrophoresis. SDS-polyacrylamide gel electrophoresis was done in accordance with the procedures of Laemmli (9). The two-dimensional system of O'Farrell et al. (10) involving nonequilibrium pH gradient electrophoresis (NEPHGE) and SDS-polyacrylamide gel electrophoresis was used. Cell-free protein translation products were added into lyophilized sample buffer (9.5 M urea, 5% β-mercaptoethanol, 0.25% SDS, 1% Nonidet P-40, 1% ampholines [pH 3.5 to 10]). The samples were applied onto the acidic end of the NEPHGE gel. The NEPHGE gel contained 2% ampholines (pH 3.5 to 10); electrophoresis was run for 5 h at 500 V. The second-dimensional separation was done by electrophoresis in SDS-containing polyacrylamide gels (10%). Radioactive proteins were visualized by fluorography with En^{3}Hance (New England Nuclear Corp., Boston, Mass.).

RESULTS

Time course of induction of the antiviral state. Monolayer A/J and A2G cells were treated with 100 reference U of IFN-β per ml for different times before infection with VSV or influenza A virus WSN. A 3-h IFN treatment resulted in a 2-log reduction of VSV yields in both A/J and A2G cells (Fig. 1A). Prolonged IFN incubation of the cells caused only little further reduction of VSV yields. In contrast to the inhibition of VSV replication in both cell lines, the replication of influenza virus was efficiently inhibited by IFN only in A2G cells and not in A/J cells (Fig. 1B). After a 3-h incubation of A2G cells with IFN, influenza virus yields were reduced about 3 logs. Further IFN exposure reduced the yields only slightly. In IFN-treated A/J cells, however, influenza virus yields were reduced just over 1 log even after a 9-h incubation. Similarly, *Mx*-linked IFN efficiency in the inhibition of avian influenza A virus (strain A/Turkey/England/63) has been found in mouse peritoneal macrophages (1).

Translation of IFN-induced mRNAs. Five-day-old monolayers of A/J or A2G cells were incubated with or without 400 reference U of IFN-β per ml in serum-free Dulbecco MEM for 3 h. The polysome-bound mRNAs were isolated and used to direct protein synthesis in the rabbit reticulocyte lysate cell-free translation system. The [^{35}S]methionine-labeled proteins synthesized in vitro with mRNAs from control and IFN-treated cells were compared after two-dimensional gel electrophoresis (Fig. 2).

Treatment of A/J and A2G cells with IFN resulted in the synthesis of new mRNAS. These

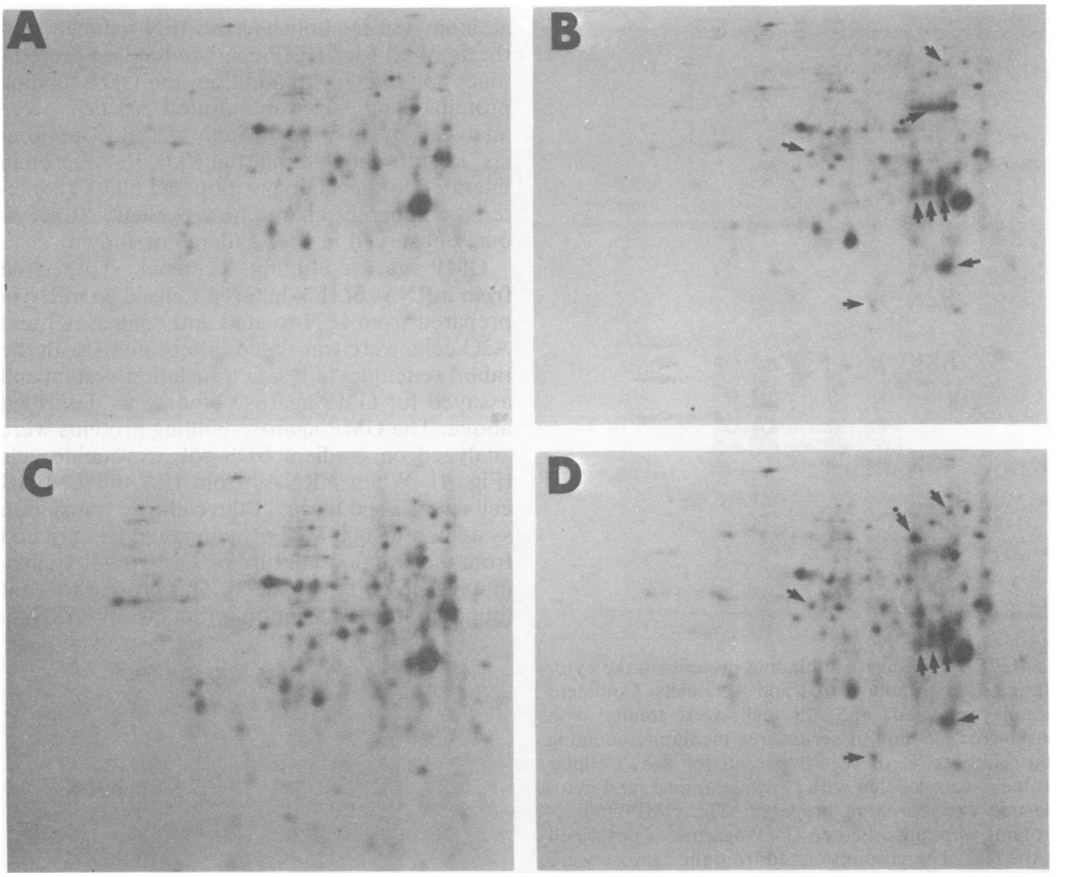

FIG. 2. Cell-free translation products of mRNA from control and IFN-treated A/J and A2G cells. Monolayers of A/J and A2G cells were treated with serum-free medium or serum-free medium containing 400 reference U of IFN-β per ml for 3 h at 37°C. Cells were harvested, and the polysomal mRNAs were extracted and used to direct protein synthesis in a rabbit reticulocyte lysate protein synthesis system. The [^{35}S]methionine-labeled proteins were separated in two-dimensional gels and visualized by fluorography. (A) Noninduced A/J cells; (B) IFN-induced A/J cells; (C) noninduced A2G cells; (D) IFN-induced A2G cells. Arrows indicate the positions of proteins translated from IFN-induced mRNAs present in both A/J and A2G cells. Arrows with asterisks indicate the proteins translated from IFN-induced mRNAs present in either A/J or A2G cells. The acidic side is on the left.

new mRNAs directed the cell-free synthesis of eight proteins that were not detected with mRNA isolated from control cells. A/J and A2G cells contained seven of these proteins with MWs of 85,000, 50,000, 45,000, 44,000, 43,000, 30,000, and 25,000 in common. The eighth new protein was different in the two cell lines; a 72,000-MW protein was observed with A2G mRNA, and a 65,000-MW protein was observed with A/J mRNA. The 65,000-MW protein synthesized from mRNA of IFN-induced A/J cells appeared in repeated experiments as a diffuse band rather than a spot (Fig. 2B). Upon isoelectric focusing, it characteristically spread over about 0.3 pH units. The pI of this protein was 5.8 to 6.1 (data not shown). Within the limits of detection, the 65,000-MW protein could not be observed in translation products of mRNAs from IFN-induced A2G cells. The 72,000-MW protein synthesized from mRNA of IFN-induced A2G cells was absent in the translation product when mRNAs from IFN-induced A/J cells or control cells was used. This protein, with a pI of 6.0, was slightly more basic than a nearby background protein (Fig. 2D). Purified IFN, consisting of a mixture of IFN-α and IFN-β, induced the same set of mRNAs (data not shown). Therefore, IFN type I, rather than contaminating materials, was the inducing agent.

GMP-agarose-binding proteins in cytoplasms of IFN-treated A/J and A2G cells. Cheng et al. (1b) recently described an IFN-induced human fibroblast protein with an MW of 67,000. This human protein also had an apparent charge heterogeneity as detected in two-dimensional gel electro-

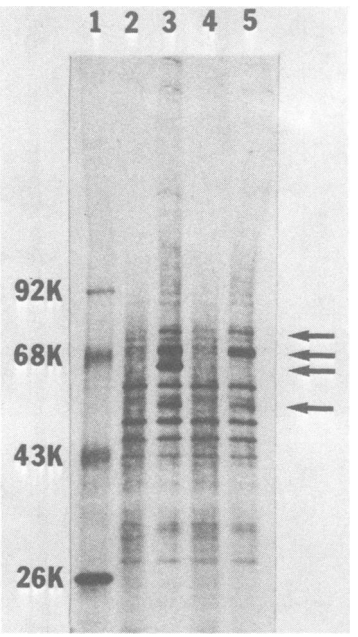

FIG. 3. GMP-agarose-binding proteins in the cytoplasms of IFN-induced A/J and A2G cells. Confluent monolayers of A/J and A2G cells were treated with serum-free medium or serum-free medium containing 400 reference U of IFN-β per ml for 4 h. Cellular proteins were labeled with [^{35}S]methionine, and cytoplasmic extracts were prepared. The GMP-binding-proteins were adsorbed on GMP-agarose as described in the text. The proteins eluted from the agarose were separated on 5.5 to 14% SDS gradient gels and visualized by fluorography. Lane 1, Protein MW standards; lane 2, noninduced A/J cells; lane 3, IFN-induced A/J cells; lane 4, noninduced A2G cells; lane 5, IFN-induced A2G cells. Arrows indicate the positions of the IFN-induced proteins.

phoresis. The pH spread for the human 67,000-MW protein was over 0.2 units. The main characteristic of the human protein was its strong affinity to guanylate residues. We therefore looked for differences among the GMP-agarose-binding proteins induced by IFN in A/J and A2G cells. The cells were treated with 400 reference U of IFN-β per ml for 4 h, and the newly synthesized proteins were labeled with [^{35}S]methionine. Cytoplasmic proteins were prepared from A/J and A2G cells and assayed for binding to GMP-agarose. The bound and subsequently eluted proteins were separated on SDS-polyacrylamide gels (Fig. 3). IFN treatment (Fig. 3, lane 3) induced a 65,000-MW protein with guanylate-binding activity in A/J cells but not in A2G cells (Fig. 3, lane 5). Four other IFN-induced GMP-agarose-binding proteins with MWs of 78,000, 71,000, 70,000, and 55,000 were found in IFN-treated cells from A/J and A2G mice. Treatment of the cell monolayers with actinomycin D abolished the IFN-induced synthesis of all four GMP-agarose-binding proteins (data not shown). In addition, the GMP-binding proteins synthesized in cultured A/J cells were analyzed by two-dimensional gel electrophoresis. Results showed that the 65,000-MW protein migrated to the same position and had a characteristic apparent charge heterogeneity as previously observed in Fig. 2 (data not shown).

GMP-agarose-binding proteins synthesized from mRNAs of IFN-induced Cells. The mRNAs prepared from IFN-treated and control A/J and A2G cells were translated into proteins with the rabbit reticulocyte lysate translation system and assayed for GMP-agarose binding as described above. The GMP-agarose-binding proteins were analyzed on gradient SDS-polyacrylamide gels (Fig. 4). When mRNAs from IFN-induced A/J cells were used to direct the cell-free translation system, a 65,000-MW protein could be enriched from the reaction mixture by GMP affinity chromatography (Fig. 4, lane 2). This protein was not found in the translation products of mRNAs

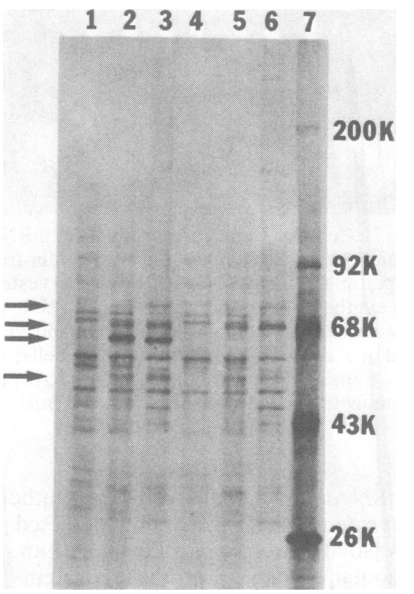

FIG. 4. In vitro-synthesized GMP-agarose-binding proteins coded for by mRNAs from IFN-treated A/J and A2G cells. mRNAs from control or IFN-treated A/J (lanes 1 and 2, respectively) or A2G (lanes 4 and 5, respectively) cells were translated in the rabbit reticulocyte lysate translation system. Portions of translation products with 2×10^6 cpm were assayed for GMP-agarose-binding activity. The bound and subsequently eluted proteins were separated on a 5.5 to 14% SDS gradient gel and visualized by fluorography. In vivo-labeled GMP-agarose-binding proteins from IFN-treated A/J (lane 3) and A2G (lane 6) cells were run on the same gel for comparison. Lane 7, Protein MW standards. Arrows indicate the positions of the IFN-induced proteins.

from IFN-induced A2G cells (Fig. 4, lane 5). Minor GMP-agarose-binding proteins with MWs of 78,000, 71,000, 70,000, and 55,000 were also detected in cell-free translation products of mRNAs from IFN-induced A/J and A2G cells. The guanylate-binding proteins synthesized in the cell-free system corresponded in number and MW to their in vivo counterparts (Fig. 4, lanes 3 and 6, respectively). In addition, this study suggests that the GMP-agarose-binding proteins did not arise from post-translational modification.

DISCUSSION

IFN induced several new mRNAs in cultured mouse embryo cells. These mRNAs could be translated into eight proteins with a rabbit reticulocyte lysate in vitro translation system. Experiments with highly purified IFN-α/β indicated that all of these mRNAs were induced by IFN type I rather than by contaminating materials. Although treatment of both A/J and A2G cells with IFN resulted in the appearance of at least eight newly induced mRNAs, a different induced mRNA was found in each. An mRNA coding for a 72,000-MW protein was found in IFN-induced A2G cells but not in IFN-induced A/J cells. In contrast, IFN-induced A/J cells contained an mRNA coding for a 65,000-MW protein that was absent in IFN-induced A2G cells (Fig. 2).

Horisberger et al. (7) described a 72,500-MW protein with a pI of 6.3 in cytoplasmic extracts of IFN-treated A2G cells but not in IFN-treated A/J cells. Genetic studies have demonstrated that IFN induction of this protein and the *Mx* resistant phenotype segregate together, suggesting that both traits are likely controlled by the same allele. The unique IFN-induced mRNA of A2G cells described in this report may code for the 72,500-MW protein of *Mx*-bearing cells because this mRNA directed the cell-free synthesis of a protein with properties very similar to those of the cytoplasmic extract 72,500-MW protein described by Horisberger et al. (7).

The unique characteristic of the 65,000-MW protein synthesized from an mRNA of IFN-induced A/J cells was its ability to bind to GMP-agarose. This property not only allowed the enrichment of this protein from the translation mixture by affinity chromatography (Fig. 4) but also facilitated the purification of the authentic protein from the IFN-induced A/J cells (Fig. 3). A2G cells did not have this protein or its mRNA.

Cheng et al. (1b) described an IFN-induced 67,000-MW protein of unknown function in human fibroblast cells which had a strong affinity for GMP-agarose and spread over 0.2 pH units during isoelectric focusing. The IFN-induced 65,000-MW protein of mouse cells that we described in this report had not only a similar MW and a similar GMP-agarose-binding activity but also had a pI of 0.3 pH units (Fig. 2B). It may well be that the mouse and human GMP-binding proteins have similar but so far unknown functions.

Other IFN-induced mRNAs common in both A/J and A2G cells were found with the rabbit reticulate lysate cell-free translation system. They varied in MW from 80,000 to 30,000. Four additional proteins were identified after enrichment by GMP-agarose chromatography. Whether any of these proteins are identical to those detected on two-dimensional gels has not been determined.

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