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Combined Action of Mouse α and β Interferons in Influenza Virus-Infected Macrophages Carrying the Resistance Gene *Mx*

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In mice, the combined action of α and β interferons (IFNs) against influenza viruses is modulated by the host gene *Mx*. High concentrations of IFN fail to prevent efficiently the replication of influenza A virus in cultured macrophages lacking the gene *Mx*, whereas cultured macrophages carrying *Mx* develop strong antiviral activity even at low concentrations of IFN. Several steps in the replication cycle of influenza virus were compared in Mx/Mx and +/+ mouse macrophages treated with IFN-α+β. Uncoating was not affected. A twofold reduction in the accumulation of primary transcripts was observed in IFN-treated macrophages at the highest concentration of IFN regardless of the genetic constitution of the host cell. No evidence was obtained for inhibition of influenza virus translation in macrophages which lacked *Mx* when treated with IFN-α+β. In contrast, a marked shut-off of influenza virus polypeptide synthesis occurred in *Mx*-bearing macrophages treated with these IFNs, although the primary transcripts were active in directing the synthesis of viral polypeptides in a cell-free system. We concluded that a specific inhibitory mechanism for influenza virus translation was induced by IFN-α+β in macrophages bearing the resistance gene *Mx*.

Interferon (IFN) was discovered by Isaacs and Lindenmann (19) during studies undertaken to determine the mechanism by which inactivated influenza virus interferes with the growth of infectious influenza virus. Despite the considerable length of time since that discovery, the mechanism of action of IFN against influenza viruses is still poorly understood. One report has suggested that primary transcription of influenza virus was inhibited by IFN in chick embryo fibroblasts (5) and another that in chick embryo fibroblasts and mouse L cells IFN does not act at the level of primary transcription but at a subsequent step (32).

We have reinvestigated the problem in mouse cells in which the activity against influenza A viruses induced by IFN-α+β is controlled by the host gene *Mx* (13). The gene *Mx* originates from the inbred mouse strain A2G, which has inborn resistance to influenza virus infection (28). Very efficient antiviral activity is induced by IFN-α+β in macrophages and in embryonic cells bearing the gene *Mx*, whereas the replication of influenza A viruses is poorly inhibited by IFN in mouse cells lacking *Mx* (2, 13; for a review, see reference 12). We therefore examined the effect of these IFNs on several steps of influenza virus replication at the molecular level.

Influenza virus is an enveloped negative-strand RNA virus with a segmented genome. Eight viral genes code for at least 10 different proteins. The virion is made up of seven proteins: the nucleoprotein (NP) and three large polypeptides (PB_1, PB_2, and PA), which are associated with the nucleocapsid core, the matrix protein (M_1), which lies within the lipid envelope, and two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which are situated at the outer surface of the envelope. At least three virus-coded nonstructural proteins are found in infected cells, but their functions are unknown (23, 25, 34). To start its infectious cycle, the influenza virus must uncoat to render its genome functional. The nucleus is most likely to be the site for transcription of the input virus genome (4, 15, 29). The virus genome is transcribed into mRNAs which are incomplete copies lacking sequences complementary to the 5'-terminal 16 nucleotides of the viral RNA (33, 35). The viral mRNAs have a capped oligonucleotide derived from the host cell (10 to 15 bases long) at their 5' end (6, 8, 21) and a polyadenylate [poly(A)] tail at their 3' end (9, 11, 14). Infected cells contain a second population of complementary RNAs (cRNAs) which are complete copies of each viral RNA segment and may act as templates for progeny viral RNA synthesis (14). Viral protein synthesis is required for secondary transcription and amplification of the genome (4, 29), and virus particles assemble at the cellular membrane by a budding process. Previous experiments with IFN-treated cultured macrophages bearing the gene *Mx* indicated that influenza virus replication was blocked at an early stage of replication after viral attachment and penetration (17). In the present study, we examined uncoating, primary transcription, and translation in an attempt to find a virus-specific function that is blocked in IFN-treated, *Mx*-bearing macrophages.

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MATERIALS AND METHODS

Mice and cell cultures. A2G mice, homozygous for the dominant resistance gene *Mx* (genotype Mx/Mx), were obtained from Olac (Bicester, England). BALB/c mice lacking *Mx* (genotype +/+) were bred locally. Thioglycolate-induced peritoneal macrophages from 2- to 3-month-old mice were harvested and cultured for 2 to 3 weeks as previously described (13).

IFN. Mouse IFN-α+β (a mixture of α and β IFNs), induced by Newcastle disease virus in C-243 sarcoma cells, were partially purified to 10^7 U/mg of protein as previously described (36) (a gift from I. Gresser, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France). Cultured macrophages were treated for 18 h with 40 or 400 NIH U of IFN per ml in RPMI 1640 medium containing 3% fetal calf serum before virus infection.

Virus and infection. M-TUR, an avian influenza A virus strain derived from strain A/Turkey/England/63, was adapted to grow in cultured mouse peritoneal macrophages (27). Stock virus was grown in 10-day-old chicken eggs at titers of

$10^{8.4}$ to $10^{8.5}$ PFU/ml as measured on primary calf kidney cell cultures. Cultured macrophages were infected for 30 min at 4°C with 10 PFU/cell for measuring uncoating or at 20°C with 100 PFU/cell for the experiments on transcription and translation. The macrophage cultures were then washed with phosphate-buffered saline and maintained at 37°C in RPMI 1640 medium containing 5% fetal calf serum. [5,6-^{3}H]uridine-labeled M-TUR virus was grown in primary calf kidney cell cultures infected at 1 PFU/cell. At 2 h after infection, the growth medium was replaced by fresh medium containing 75 μCi of [5,6-^{3}H]uridine (52 Ci/mmol; Amersham International) per ml. The labeled virus released into the medium was harvested 24 h after infection, purified by centrifugation on a sucrose cushion, and banded on a tartrate gradient as previously described (16).

Uncoating. Essentially we followed the procedure of Koff and Knight (20). Macrophage cultures were infected with 10 PFU of [5,6-^{3}H]uridine-labeled M-TUR virus per cell. At various times after infection, cultures were put on ice and washed twice with phosphate-buffered saline. Cells were detached with a rubber policeman and pelleted at 180 × *g* for 5 min. The macrophages were suspended in 850 μl of Hanks balanced salt solution containing 0.02% bovine serum albumin, and the cell suspension was frozen and thawed three times. The cell suspension was split into three aliquots of 250 μl each. One aliquot was diluted with 250 μl of water and precipitated with 5% trichloroacetic acid for measuring total radioactivity. A 250-μl amount of a mixture of RNases A and T_1 (Serva) was added to the second aliquot, giving final concentrations of 10 μg of RNase A per ml and 50 U of RNase T_1 per ml. The third aliquot was adjusted to 0.8% Triton N-101 (Sigma Chemical Co.) before the RNase mixture was added. Aliquots 2 and 3 were incubated for 30 min at 37°C and then precipitated with 5% trichloroacetic acid. The acid-insoluble material was collected on filter disks (Whatman GF/C), washed with ethanol, and dried. Protein and RNA were solubilized from the disks with 0.1 ml of water and 1 ml of Soluene 100 (Packard Instruments) for 16 h at 20°C. Ten milliliters of neutralized Aquasol-2 (50 ml of 1 N HCl added to 1 liter of Aquasol-2; New England Nuclear Corp.) was added to each sample for scintillation counting.

Extraction of RNA. Macrophages of radiolabeled M-TUR virus were disrupted with 1% sodium dodecyl sulfate (SDS) containing 100 μg of predigested proteinase K (Merck) per ml. After 30 min at 37°C, the mixtures were extracted three times with 1 volume of phenol saturated with 0.1 M Tris hydrochloride (pH 9) containing 0.1% hydroxyquinoline. The nucleic acids were precipitated from the aqueous phase by adding NaCl to 0.1 M and 2 volumes of ethanol at −20°C. The precipitated RNA was washed with 70% ethanol containing 0.1 M NaCl.

RNA-RNA hybridization and analysis. Cellular RNA (50 to 300 μg) mixed with [5,6-^{3}H]uridine-labeled virion RNA in excess (greater than 20-fold excess with regard to cRNA) was denatured at 45°C for 30 min in 9 volumes of dimethyl sulfoxide. The mixture was then hybridized for 16 h at 37°C as previously described (14). Two different methods were used for quantitative analysis of hybrids. Method 1: hybrids were dissolved in 0.5 M NaCl–1 mM EDTA–10 mM Tris hydrochloride (pH 7.6)–0.5% SDS. Poly(A)-containing hybrids were purified by three cycles of chromatography on oligodeoxythymidylate-cellulose (3). Recovery reached 93 to 98% of the input radioactivity. Hybridization with RNA from uninfected macrophages gave less than 6% residual radioactive RNA in the poly(A)-containing fraction relative to that in infected macrophages. Method 2: hybrids were ethanol-precipitated and dissolved in 100 mM NaCl–10 mM sodium acetate (pH 4.5)–0.5 mM $ZnSO_4$. Single-stranded RNA was digested with 10 to 15 U of nuclease S1 (Boehringer) per μg of RNA for 4 h at 37°C. The digestion was stopped by adjusting the mixture to 1% SDS and 1 mM EDTA. Hybrids were precipitated with ethanol and separated in a slab gel containing 4% acrylamide–0.2% bisacrylamide for 15 h at 100 V, essentially as described before (14). The gels were processed for fluorography and exposed to X-ray film (Eastman Kodak Co.) for 1 to 3 weeks. Bands of double-stranded viral RNA were cut and counted directly. Background levels were determined with samples from uninfected macrophages.

Translation of primary transcripts and protein synthesis. In vivo translations were carried out in cultures of macrophages infected at a multiplicity of approximately 100 PFU/cell and maintained in the presence of 100 μg of cycloheximide per ml for 4 h after infection. The drug was then removed, and the cells were washed five times with Hanks balanced salt solution and then labeled for 15 min at 37°C with 100 μCi of [^{35}S]methionine (1,300 to 1,500 Ci/mmol; Amersham) per ml in Hanks balanced salt solution. Cells were washed with ice-cold buffer containing 1.5 mM KCl, 2.5 mM $MgCl_2$, and 5 mM Tris hydrochloride (pH 7.4) and then lysed for 5 min in the same buffer containing 1% Triton X-100 and 1% sodium deoxycholate. The extract was centrifuged for 5 min at 2,300 × *g*, and the supernatant was collected. Total radioactivity and protein content were determined. Proteins were analyzed by separation in two dimensions as previously described (16), combining nonequilibrium pH gradient electrophoresis with SDS-polyacrylamide gel electrophoresis (30). Cell-free translations were carried out with 20 μg of cellular RNA added to 10 μl of a rabbit reticulocyte lysate cell-free translation system (Amersham, no. N.90) containing 1.2 to 3.6 μCi of [^{35}S]methionine per μl. Samples were incubated at 32°C for 90 min. Protein synthesis was measured by the procedure indicated by the manufacturer, and the radioactive polypeptides were analyzed on two-dimensional gels by the procedure described above.

RESULTS

Effect of IFN-α+β on uncoating of influenza virus. Macrophages from resistant A2G mice (Mx/Mx) and from sensitive BALB/c mice (+/+), cultivated for 2 to 3 weeks, are equally effective for the replication of influenza virus M-TUR (13). The influenza virus yields in this study were reduced 5-fold and 45-fold, respectively, by treatment with IFN at 40 U/ml and 7-fold and 800-fold, respectively, by treatment at 400 U/ml in macrophages lacking or carrying the gene *Mx* (Table 1). All subsequent experiments were done with 40 and 400 U of IFN per ml.

For measuring the effect of IFN on uncoating, cultivated macrophages were infected at 4°C with 10 PFU of M-TUR virus labeled with tritiated uridine per cell. Virus uncoating was measured 5, 30, 60, and 120 min after the shiftup of the

TABLE 1. Effect of IFN on reduction of virus yield in macrophages infected with influenza A virus M-TUR

IFN Concn (U/ml)	Virus yield (PFU/ml) in macrophages with genotype:	
	+/+	Mx/Mx
0	17×10^6	15×10^6
40	3.7×10^6	3.3×10^5
400	2.3×10^6	1.8×10^4

TABLE 2. Effect of IFN on uncoating of influenza virus

Time after infection (min)	% (±SD)[a] RNase-resistant viral RNA in macrophages at indicated IFN concn (U/ml)					
	+/+			Mx/Mx		
	0	40	400	0	40	400
5	93 ± 6	87 ± 1	88 ± 1	98 ± 1	96 ± 1	98 ± 2
30	71 ± 2	72 ± 2	73 ± 2	74 ± 5	70 ± 7	68 ± 7
60	57 ± 4	54 ± 6	56 ± 1	55 ± 2	53 ± 5	59 ± 2
120	51 ± 2	52 ± 2	56 ± 4	55 ± 2	54 ± 1	50 ± 7

[a] Average of two independent experiments.

cultures to 37°C. Macrophages were harvested, permeabilized by a cycle of freeze-thawing, and treated with a mixture of RNases. The percentage of radioactive viral RNA accessible to RNase digestion was taken as a measure of uncoating (20). The conditions for RNase treatment were chosen so that freeze-thawed purified virions remained resistant to digestion (95% of the radioactivity was trichloroacetic acid insoluble) and viral RNA was digested to a background level (2 to 3% of total radioactivity) after detergent treatment of purified virions or permeabilized infected cells. Without the addition of the RNases, the viral RNA was stable in permeabilized cells for at least 30 min at 37°C.

The kinetics of uncoating were essentially the same in both cell types (+/+ and Mx/Mx), and they were not influenced by IFN treatment (Table 2). Uncoating occurred within the first hour after the attachment period. About half of the virions that penetrated the cells became uncoated, whereas the other half remained RNAse resistant and stably associated with the infected cells for at least 1 h after termination of the uncoating process. Comparable results have been obtained by others (20) and in our laboratory (data not shown) with cultivated cells of different origin and different strains of influenza virus.

Effect of IFN on primary transcription. In cells infected with influenza virus in the presence of concentrations of cycloheximide sufficient to inhibit protein synthesis, virus-specific primary transcription is permitted, but not replication or amplification of the genome (4, 14, 29). Such synthesis is catalyzed by virus-associated enzymes and proceeds unaltered for at least 5 h, and the products are mRNAs complementary to their respective genome segments (14).

We measured the effect of IFN-$\alpha+\beta$ on transcription in cultured macrophages treated with 40 or 400 U of IFN per ml for 18 h. Control cultured macrophages were left untreated. RNA was extracted from cultures that had been incubated with cycloheximide (100 μg/ml) for 30 min before and 4 h after infection with influenza virus M-TUR at a multiplicity of approximately 100 PFU/cell. The RNA was annealed with [5,6-^{3}H]uridine-labeled virion RNA (12,000 cpm/μg of virion RNA), and the poly(A)$^+$, virus-specific mRNAs, annealed with radioactive viral RNA, were selected by oligodeoxythymidylate-cellulose chromatography as described above. Quantitative analysis of primary transcription by this method indicated that an average of 8,000 poly(A)$^+$ genome copies per control macrophage were accumulated 4 h after infection in both +/+ and Mx/Mx macrophages. IFN treatment decreased the amounts of primary transcripts to the same extent in both types of macrophages (Table 3). The amounts represented about 65 and 40% of the control values at 40 and 400 U of IFN per ml, respectively.

This effect of IFN on in vivo primary transcription could be due to a general decrease in the accumulation of all primary transcripts or to a selective effect on the transcription of one or several genes. To distinguish between these possibilities, we analyzed the relative amounts of cRNAs transcribed from each viral gene. The RNA extracted from infected macrophages was annealed to [5,6-^{3}H]uridine-labeled virion RNA. The hybrids were digested with S1 nuclease, and the double-stranded RNAs were separated by polyacrylamide gel electrophoresis (14) and visualized by fluorography (4). The transcription products of genes 1 through 3 (corresponding to the three large P polypeptides) were not resolved in this type of analysis. For unknown reasons, gene 5 (coding for NP) seemed to be transcribed more efficiently than the other genes. The splitting of bands corresponding to genes 7 and 8 indicated that some complete RNA transcripts might have been synthesized in the presence of cycloheximide in higher amounts than have been observed by others (4, 29). The limited amounts of cRNA obtainable from infected macrophage cultures did not allow detailed characterization of these RNAs. Infected Mx/Mx and +/+ mouse macrophages gave essentially the same patterns for synthesis of cRNAs (Fig. 1). The amounts of individual transcripts were decreased by IFN to a similar extent, and we found no evidence for selective inhibition of a particular transcript. Radioactive bands were cut out for quantification by scintillation counting. The sum of the radioactive cRNAs was measured and expressed as a percentage of the controls. The average values of two independent experiments are given in Table 3. The gene *Mx* did not

TABLE 3. Quantitative effect of IFN on transcription and translation of influenza virus

Activity measured	Accumulation or synthesis (% of control ± SD)[a] in macrophages at indicated IFN concn (U/ml)					
	+/+			Mx/Mx		
	0	40	400	0	40	400
Primary transcription						
Poly (A)$^+$ RNAs	100	64 ± 9	45 ± 7	100	68 ± 6	36 ± 6
cRNAs	100	58 ± 11	41 ± 15	100	60 ± 14	49 ± 11
Translation						
In vivo	100	65 ± 16	55 ± 14	100	6 ± 1	1
In vitro	100	66 ± 11	58 ± 15	100	66 ± 6	53 ± 6

[a] Average of two independent experiments.

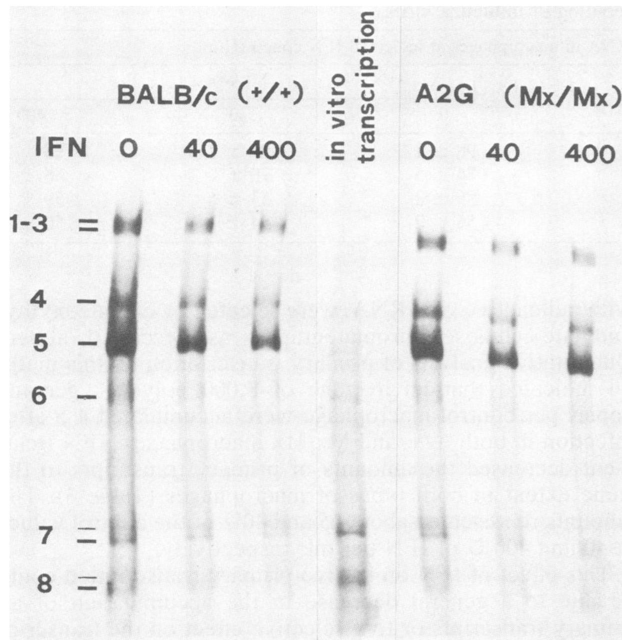

FIG. 1. Composition of cRNAs isolated from influenza virus-infected macrophages treated or not treated with IFN. Infected macrophages were treated as described in the text. The labeled virion RNA shown in the central slot was hybridized to transcripts synthesized in vitro by the virion-associated transcriptase. The reaction mixture contained 80 mM Tris hydrochloride (pH 8), 30 mM CH_3COOK, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.4 mM ApG, 0.8 mM ATP, 0.4 mM CTP, 0.4 mM UTP, 20 μM [^{3}H]GTP (1 mCi/mmol), 0.05% Nonidet P-40, 2 mg of Macaloid per ml, and purified influenza virus (approximately 1 mg/ml) and was incubated at 32°C for 90 min. All samples were run in the same gel. The film was exposed for 9 days, with the exception of the central slot, which was exposed for 21 days. Virus gene numbers are indicated to the left.

modulate the inhibitory action of IFN-α+β on primary transcription, as measured by this method. The accumulation of cRNAs represented about 60 and 45% of the control values at 40 and 400 U of IFN per ml, respectively. The extent of this effect was in good agreement with the reduction of accumulation of poly(A)$^+$ viral mRNAs (Table 3). We deduced from these experiments that *Mx* did not influence the level of primary transcription in macrophages treated with IFN-α+β.

Effect of IFN on in vivo and in vitro translation. Macrophage cultures were treated with 40 or 400 U of IFN-α+β per ml for 18 h or left untreated. They were then infected at a multiplicity of 100 PFU of influenza virus M-TUR per cell in the presence of 100 μg of cycloheximide per ml. These conditions permitted primary transcription, but protein synthesis was inhibited by 98%. For in vivo translation, proteins were labeled with [^{35}S]methionine during a 15-min period immediately after removal of the drug, since by this procedure viral polypeptides can be synthesized from the mRNAs produced by primary transcription (22). The synthesis of the viral polypeptides NP, M_1, and NS_1 was detected with certainty in infected macrophages (Fig. 2c and d), since these viral polypeptides were absent from uninfected cells and since they did not overlap major host polypeptides (arrows, Fig. 2a and b). At the highest concentration of IFN (400 U/ml), viral protein synthesis was slightly diminished in +/+ macrophages (Fig. 1e). In contrast, NP, M_1, and NS_1 were barely visible in IFN-treated Mx/Mx macrophages (Fig. 1f). The spots corresponding to NP, M_1, and NS_1 were cut out and quantified by scintillation counting. The sum of the radioactivity was taken as a measure of in vivo translation; the reference value was the amounts synthesized in infected macrophages not treated with IFN. IFN decreased the in vivo translation of viral polypeptides in infected +/+ macrophages (Table 3) in a concentration-dependent fashion. The extent of in vivo synthesis represented 66% of the control values at 40 U and 55% at 400 U of IFN per ml (average of two independent experiments). This reduction of in vivo synthesis corresponded to the reduced levels of primary transcription measured at these concentrations of IFN (Table 3). It seemed, therefore, that IFN did not exert a specific inhibitory effect on the translation of virus-specific mRNAs in +/+ macrophages. On the other hand, the in vivo translation of viral primary mRNAs was markedly inhibited in Mx/Mx macrophages treated with IFN (Table 3); it was reduced approximately 15-fold at 40 U/ml and 100-fold at 400 U/ml.

One obvious reason for this effect could be that the viral mRNAs were defective in some way and incapable of directing viral protein synthesis. To examine this possibility, we extracted the RNA at the end of the primary transcription period (4 h after infection in the presence of 100 μg of cycloheximide per ml). RNA was added to an mRNA-dependent translation system (7, 31) from rabbit reticulocyte lysates. Protein synthesis was carried out in the presence of [^{35}S]methionine, and the products were separated on two-dimensional gels. Successful translation of NP, M_1, and NS_1 polypeptides was demonstrated with RNA from infected Mx/Mx macrophages treated with 400 U of IFN per ml (Fig. 3d). Essentially, Mx/Mx and +/+ macrophages gave comparable results in this type of experiment. The amount of NP synthesized was greater than those of M_1 and NS_1, which was in accordance with the relative accumulation of transcripts for their respective genes 5, 7, and 8 (Fig. 1). Quantification of in vitro translation (the sum of NP, M_1, and NS_1) demonstrated that viral polypeptide synthesis in vitro was reduced to comparable levels in transcripts isolated from either Mx/Mx or +/+ macrophages (Table 3). This reduction reflected the smaller accumulation of primary transcripts at the corresponding IFN concentrations, as determined by direct measurement (Table 3). We deduced from these experiments that primary mRNAs for NP, M_1, and NS_1 synthesized in IFN-treated Mx/Mx macrophages were fully active in directing viral polypeptide synthesis in vitro, even though they were poorly translated in vivo.

DISCUSSION

Influenza A viruses are poorly inhibited by IFN in mouse cells that do not carry the resistance gene *Mx* (1, 2, 13, 32). For instance, in cultivated macrophages lacking *Mx*, a sevenfold reduction in virus yield (influenza A virus M-TUR) was obtained at 400 U of a mixture of α and β IFNs per ml. However, the same concentration of IFN caused an 800-fold reduction in virus yield in cultured macrophages bearing *Mx*. The extent of this antiviral effect allowed us to investigate the mechanism of action of IFN in cultured macrophages against influenza A virus infection in relation to the *Mx* genotype at the molecular level. The mechanism discussed below is likely to be valid for cultivated cells other than macrophages, since *Mx* gene expression has also been

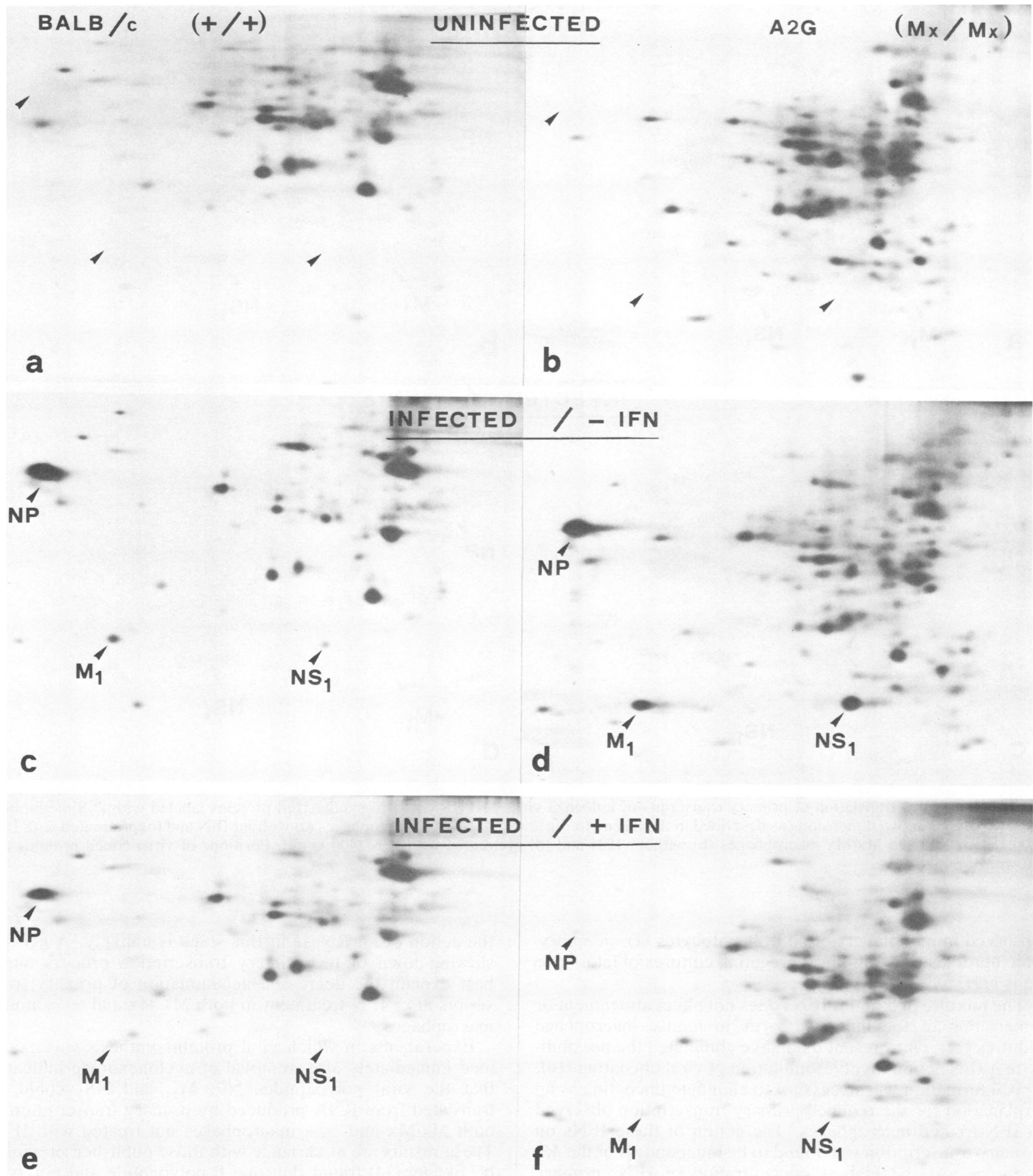

FIG. 2. In vivo translation of primary transcripts of influenza virus. Cellular extracts were prepared as described in the text and the proteins were separated by nonequilibrium pH gradient electrophoresis in the first dimension (right [anode] to left [cathode]) and then by SDS-polyacrylamide gel electrophoresis in the second dimension (top to bottom), followed by fluorography. (a, c, and e) +/+ macrophages; (b, d, and f) Mx/Mx macrophages. (a and b) No IFN treatment, no infection; (c and d) no IFN treatment, infection with influenza virus; (e and f) 400 U of IFN per ml and infection with influenza virus. Positions of virus-coded proteins are shown.

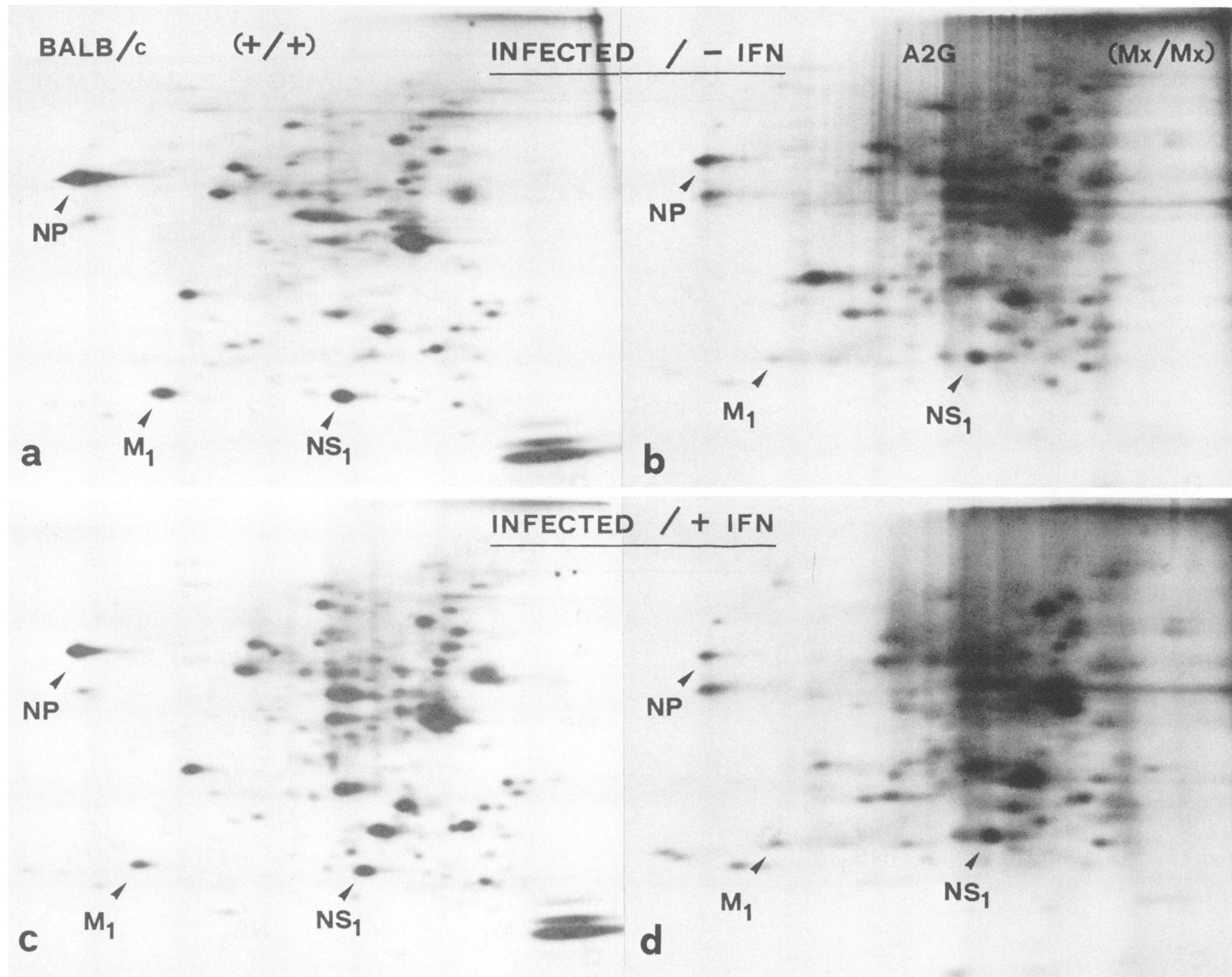

FIG. 3. In vitro translation of primary transcripts of influenza virus M-TUR. In vitro-synthesized proteins labeled with [^{35}S]methionine were separated in two dimensions as described in the legend to Fig. 2. Infected +/+ macrophages (a) without IFN and (c) pretreated with IFN (400 U/ml). Infected Mx/Mx macrophages (b) without IFN and (d) pretreated with IFN (400 U/ml). Positions of virus-coded proteins are shown.

observed in monolayers of adult hepatocytes (1), in embryonic fibroblasts (2), and in aggregating cultures of fetal brain cells (12).

The mixture of α and β IFNs does not affect attachment or penetration of the influenza virus in mouse macrophage cultures (17). Our present data have eliminated the possibility that IFN action involves inhibition of viral uncoating (10). This information was necessary to eliminate uncoating as an explanation for the reduced primary transcription observed in IFN-treated macrophages. The action of these IFNs on primary transcription was found to be independent of the *Mx* genotype. At the highest concentration of IFN, primary transcription was reduced to about 45% of that in the controls (infected macrophages not treated with IFN), which was in good agreement with the 30 to 50% reduction found by Repik et al. (32) in similarly treated mouse L cells infected with influenza A virus WSN. The effect of IFN on primary transcription did not reflect the selective inhibition of transcription of one or a few viral genes. The integrity of the transcripts seemed to be preserved, which suggests that the action of an RNase at this stage is unlikely. A general slowing-down of the primary transcription process might best explain the decreased accumulation of primary transcripts after IFN treatment in both Mx/Mx and +/+ mouse macrophages.

Experiments in which viral protein synthesis was examined immediately after removal of cycloheximide indicated that the viral polypeptides NP, M_1, and NS_1 could be translated from RNA produced by primary transcription in both Mx/Mx and +/+ macrophages not treated with IFN. These results are at variance with those published previously. Skehel (34) found that one P polypeptide and polypeptides NP and NS_1 were detected immediately after reversal of the cycloheximide block in chick embryo cells infected with fowl plague virus. Lamb and Choppin (22) reported that the three P polypeptides and polypeptides NP, M_1, and NS_1 were detected in similarly treated chick embryo cells infected with influenza A virus NWS. These variations may be due to differences in cell type, virus strain, virus inoculum, and sensitivity of the techniques used. In +/+ macrophages, the

approximately twofold decrease in synthesis of viral polypeptides NP, M_1, and NS_1 after IFN treatment reflected quantitatively the smaller accumulation of the corresponding primary transcripts. We concluded therefore that IFN had no measurable effect on the translation of influenza viral polypeptides in +/+ macrophages. In contrast, the in vivo synthesis of NP, M_1, and NS_1 was decreased about 100-fold in Mx/Mx macrophages at the highest concentration of IFN, even though the corresponding primary mRNAs had been transcribed and were capable of directing protein synthesis in a heterologous cell-free translation system. Transfection experiments have indicated that translation of the NP and M_1 proteins does not depend on the expression of other influenza virus genes (24, 26). Therefore the inhibition of viral protein synthesis in vivo due to the concerted action of IFNs α and β and of the gene *Mx* could not be traced to a lack of demonstrable synthesis of P polypeptides and glycoproteins after reversal of the cycloheximide block. It is conceivable that the transport of primary transcripts from the nucleus to the cytoplasm might have been impaired. However, our preliminary experiments (unpublished data) showing normal accumulation of primary transcripts in the cytoplasm of IFN-treated Mx/Mx macrophages make this hypothesis unlikely. Taken together, these results suggest that the *Mx* genotype exerts a block on apparently functional viral mRNAs at the level of translation in IFN-treated Mx/Mx macrophages. This site of action may explain the very efficient antiviral activity against influenza virus elicited by IFNs α and β in cells bearing the gene *Mx*, since viral protein synthesis is necessary for replication and amplification of the genome (4, 14, 29).

Recently, Horisberger et al. (18) have found that IFN-$\alpha+\beta$ induce a unique 72,000-molecular-weight protein (p72) in *Mx*-bearing cells. Evidence suggesting the involvement of p72 in the specific antiviral state rests on the facts (i) that induction of specific antiviral activity against influenza viruses by these IFNs is correlated with the appearance of p72, (ii) that the inducibility of p72 by these IFNs cosegregates with the *Mx* genotype in congenic mice (18), and (iii) that IFN-γ does not induce p72 and fails to establish specific and efficient antiviral activity in *Mx*-bearing cells (18a; P. Staeheli, M. A. Horisberger, and O. Haller, Virology, in press). p72 does not seem to be related to the $(2'-5')(A)_n$ synthetase or to the protein kinase induced by IFN (18). If this protein acts as a discriminatory factor for influenza virus mRNA, it would explain why the replication cycle of influenza virus is so abruptly interrupted at a precise step, i.e., translation, in *Mx*-bearing cells. We are at present attempting to define the mechanism of its action. It seems possible that this system may be valuable in shedding light on the regulatory mechanisms which operate at the translational level of influenza virus mRNAs and host cell mRNAs. The *Mx* system illustrates how the action of IFN can be modulated by the genetic makeup of the target cell, resulting in a virus-specific effect. This phenomenon may have much wider significance for the mechanism of action of IFN in general.

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LITERATURE CITED

1. **Arnheiter, H., O. Haller, and J. Lindenmann.** 1980. Host gene influence on interferon action in adult mouse hepatocytes: specificity for influenza virus. Virology **103**:11–20.
2. **Arnheiter, H., and P. Staeheli.** 1983. Expression of interferon dependent resistance to influenza virus in mouse embryo cells. Arch. Virol. **76**:127–137.
3. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408–1412.
4. **Barrett, T., A. J. Wolstenholme, and B. W. J. Mahy.** 1979. Transcription and replication of influenza virus RNA. Virology **98**:211–225.
5. **Bean, W. J., and R. W. Simpson.** 1973. Primary transcription of the influenza virus genome in permissive cells. Virology **56**:646–651.
6. **Caton, A. J., and J. S. Robertson.** 1980. Structure of the host-derived sequences present at the 5′ ends of influenza virus mRNA. Nucleic Acids Res. **8**:2591–2603.
7. **Content, J., L. De Wit, and M. A. Horisberger.** 1978. Peptide mapping characterization of viral proteins generated in a cell-free coupled system for the transcription and translation of influenza virus mRNA. J. Virol. **26**:817–821.
8. **Dhar, R., R. M. Channock, and C.-J. Lai.** 1980. Nonviral oligonucleotides at the 5′ terminus of cytoplasmic influenza viral mRNA deduced from cloned complete genome sequences. Cell **21**:495–500.
9. **Etkind, P. R., and R. M. Krug.** 1974. Influenza viral mRNA. Virology **62**:38–45.
10. **Friedman, R. M.** 1977. Antiviral activity of interferon. Bacteriol. Rev. **41**:543–567.
11. **Glass, S. E., D. McGeoch, and R. D. Barry.** 1975. Characterization of the mRNA of influenza virus. J. Virol. **16**:1435–1443.
12. **Haller, O.** 1981. Inborn resistance of mice to orthomyxoviruses. Curr. Top. Microbiol. Immunol. **92**:24–52.
13. **Haller, O., H. Arnheiter, J. Lindenmann, and I. Gresser.** 1980. Host gene influences sensitivity to interferon action selectively for influenza virus. Nature (London) **283**:660–662.
14. **Hay, A. J., B. Lomniczi, A. R. Bellamy, and J. J. Skehel.** 1977. Transcription of the influenza virus genome. Virology **83**:337–355.
15. **Herz, C., E. Stavnezer, R. M. Krug, and T. Gurney.** 1981. Influenza virus, an RNA virus, synthesizes its messenger RNA in the nucleus of infected cells. Cell **26**:391–400.
16. **Horisberger, M. A.** 1980. The large P proteins of influenza A viruses are composed of one acidic and two basic polypeptides. Virology **107**:302–305.
17. **Horisberger, M. A., O. Haller, and H. Arnheiter.** 1980. Interferon-dependent genetic resistance to influenza virus in mice: virus replication in macrophages is inhibited at an early step. J. Gen. Virol. **50**:205–210.
18. **Horisberger, M. A., P. Staeheli, and O. Haller.** 1983. Interferon induces a unique protein in mouse cells bearing a gene for resistance to influenza virus. Proc. Natl. Acad. Sci. U.S.A. **80**:1910–1914.

18a. **Horisberger, M. A., P. Staeheli, and O. Haller.** 1983. Proteins induced by type I and type II interferons in mouse cells bearing the gene for influenza virus resistance, p. 251–256. *In* E. De Mayer and H. Schellekens (ed.), The biology of the interferon system 1983. Elsevier Science Publishers, Amsterdam.

19. **Isaacs, A., and J. Lindenmann.** 1957. Virus interference. I. The interferon. Proc. R. Soc. London Ser. B **147**:258–267.
20. **Koff, W. C., and V. Knight.** 1979. Inhibition of influenza virus uncoating by rimantadine hydrochloride. J. Virol. **31**:261–263.
21. **Krug, R. M., B. A. Broni, and M. Bouloy.** 1979. Are the 5′ ends of influenza mRNAs synthesized *in vivo* donated by the host cell mRNAs? Cell **18**:329–334.
22. **Lamb, R., and P. W. Choppin.** 1976. Synthesis of influenza virus proteins in infected cells: translation of viral polypeptides, including three P polypeptides, from RNA produced by primary transcription. Virology **74**:504–519.
23. **Lamb, R., and P. W. Choppin.** 1981. Identification of a second protein (M_2) encoded by RNA segment 7 of influenza virus. Virology **112**:729–737.
24. **Lamb, R. A., and C.-J. Lai.** 1982. Spliced and unspliced messenger RNAs synthesized from cloned influenza virus M DNA in an SV40 vector: expression of the influenza virus membrane protein (M_1). Virology **120**:237–256.

25. **Lazarowitz, S. G., R. W. Compans, and P. W. Choppin.** 1971. Influenza virus structural and nonstructural proteins in infected cells and their plasma membranes. Virology **46:**830–843.
26. **Lin, B.-C., and C.-J. Lai.** 1983. The influenza virus nucleoprotein synthesized from cloned DNA in a simian virus 40 vector is directed in the nucleus. J. Virol. **45:**434–438.
27. **Lindenmann, J., E. Deuel, S. Fanconi, and O. Haller.** 1978. Inborn resistance of mice to myxoviruses: macrophages express phenotype *in vitro*. J. Exp. Med. **147:**531–540.
28. **Lindenmann, J., C. A. Lane, and D. Hobson.** 1963. The resistance of A2G mice to myxoviruses. J. Immunol. **90:**942–951.
29. **Mark, G. E., J. M. Taylor, B. Broni, and R. M. Krug.** 1979. Nuclear accumulation of influenza viral RNA transcripts and the effects of cycloheximide, actinomycin D, and α-amanitin. J. Virol. **29:**744–752.
30. **O'Farrel, P. Z., H. M. Goodman, and P. H. Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12:**1133–1142.
31. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67:**247–256.
32. **Repik, P., A. Flamand, and D. H. L. Bishop.** 1974. Effect of interferon upon the primary and secondary transcription of vesicular stomatitis and influenza viruses. J. Virol. **14:**1169–1178.
33. **Robertson, J. S., M. Schubert, and R. A. Lazzarini.** 1981. Polyadenylation sites for influenza virus mRNA. J. Virol. **38:**157–163.
34. **Skehel, J. J.** 1972. Polypeptide synthesis in influenza virus-infected cells. Virology **49:**23–36.
35. **Skehel, J. J., and A. J. Hay.** 1978. Nucleotide sequences at the 5′ termini of influenza virus mRNAs and their transcripts. Nucleic Acids Res. **5:**1207–1219.
36. **Tovey, M. G., J. Begon-Lours, and I. Gresser.** 1974. A method for the scale production for potent interferon preparations. Proc. Soc. Exp. Biol. Med. **146:**809–815.