Heterogeneity of interferon mRNA species from Sendai virus-induced human lymphoblastoid (Namalva) cells and Newcastle disease virus-induced murine fibroblastoid (L) cells

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ABSTRACT

Cytoplasmic polyadenylated RNA preparations obtained from Sendai-induced human lymphoblastoid (Namalva) cells and from Newcastle disease virus (NDV)-induced murine (L) cells were denatured in 10-12.5 mM CH_3HgOH and then electrophoresed in 2% agarose tube gels containing 10 mM CH_3HgOH, the RNA eluted from gel slices and translationally active interferon mRNA species located using the Xenopus oocyte assay. The interferons synthesized were characterized as α or β types based on neutralization tests using specific antisera against human or murine interferon-α and interferon-β. At least two species of mRNA for human interferon-α and two for human interferon-β were detected in RNA from Sendai-induced Namalva cells. These are (approximate mRNA length in parentheses) α(1.3 kb), α(1.9 kb), β(1.1 kb) and β(1.9 kb). Two populations of murine interferon mRNA of lengths approximately 1.4 kb and 3 kb were detected in mRNA preparations from NDV-induced L cells by electrophoresis. However, since the translation products of each of these two populations of mRNA consist of both murine interferon-α and murine interferon-β it is likely that both the 1.4 kb and 3 kb populations contain at least one species each of murine interferon-α and murine interferon-β mRNA.

INTRODUCTION

The elucidation of the structure of human interferons and of the biochemical basis for their actions is attracting considerable attention. Human interferons are now grouped into three categories based on antigenic properties; the α or leukocyte interferons, the β or fibroblast interferons, and the γ or immune interferons (1). A similar scheme extends to the murine interferons (1, 2). Furthermore species of murine interferon-α and β display sequence relatedness to species of human interferon-α and β, respectively (3, 4, 5).

Sucrose gradient sedimentation analyses of interferon-α and

β mRNA preparations from various cell sources usually show a population of interferon mRNA which sediments at approximately 12-14S (6 - 10). Nevertheless, two distinct species of human β-interferon mRNA (β_1:0.9 kb; β_2:1.3 kb) were resolved when interferon mRNA preparations from poly(I)·poly(C)-induced human diploid fibroblasts were electrophoresed through 2% agarose-10 mM CH_3HgOH gels (11, 12). We have now used this procedure to analyse interferon mRNA preparations from human lymphoblastoid (Namalva) cells and from murine (L) cells. We report that interferon mRNA from Namalva cells can be resolved into at least 4 species (α, 1.3 kb; α, 1.9 kb; β, 1.1 kb; β, 1.9 kb). Furthermore it is likely that at least 4 species of murine interferon mRNA are present in preparations from L-cells (α, 1.4 kb; α, 3 kb; β, 1.4 kb; β, 3 kb).

MATERIALS AND METHODS

Interferon mRNA preparations.

Polyadenylated mRNA was prepared from Namalva cells essentially as described earlier (7, 8, 13). Briefly, Namalva cells in spinner culture (5 x 10^5 cells/ml in RPMI medium supplimented with 10% fetal bovine serum) were treated with bromodeoxyuridine (25 μg/ml) overnight, mixed with Sendai virus (100 hemagglutination units/ml, ∿10^6 cells/ml) and incubated at 37° for 9-10 hours. The cytoplasmic fraction was prepared by hypotonic lysis in 0.5% NP40. Polyadenylated RNA was selected by 2 cycles of oligo(dT)-cellulose chromatography or by one cycle of poly(U)-Sepharose chromatography. Polyadenylated RNA preparations from NDV-induced LPA cells were also prepared as described earlier (14). Essentially, LPA cell monolayer cultures were primed with murine interferon (100 ref. units/ml) for 2 hours, induced with NDV (multiplicity of infection: 5), and harvested 11 hours following induction. The cytoplasmic fraction was prepared by detergent lysis and polyadenylated RNA selected through 2 cycles of oligo(dT)-cellulose chromatography.

Electrophoresis of mRNA in agarose-CH_3HgOH gels.

Polyadenylated RNA (40-250 μg) was mixed with ^{32}P-labeled marker rRNA and tRNA from HeLa cells, was made 10-12.5 mM in CH_3HgOH (stock 1.0 M, Alfa Division, Ventron) in half-strength

borate buffer (full strength corresponds to: 0.1 M boric acid, 6 mM sodium borate, 1 mM EDTA, 10 mM sodium sulfate, pH 8.2) in a total volume of 50 µl and was allowed to stand at room temperature for 5 minutes. Five µl of glycerol saturated with bromophenol blue was then added and the RNA electrophoresed through a 2% agarose-10 mM CH_3HgOH cylindrical gel (0.6 cm x 11 cm) in borate buffer at room temperature (4mA/tube, 3.5 volts/cm) until the dye was close to the bottom of the tube gel (∿5 hours). After electrophoresis the gel was placed in 100 ml of a solution containing 100 mM β-mercaptoethanol and 0.01 M Tris·HCl, pH 7.4 for 45 minutes on a rotary shaker at room temperature. The gel was then manually sliced (1 mm slices), the locations of marker RNA monitored by Cerenkov counting, RNA in appropriate regions of the gel eluted by pooling adjacent (2 slices/pool) slices (11, 15), dissolved in 5 µl distilled water.

Assay of interferon mRNA activity.

Two µl aliquots of appropriate RNA samples were microinjected into ∿20 oocytes of X. laevis and the oocytes incubated in 0.2 ml Barth's medium for ∿40 hrs (12, 16). The interferon content of the oocyte incubation medium (12, 17) was estimated by a semi-micro assay on human GM2504, or GM2767 (Human Genetic Mutant Cell Repository, Camden, N. J.); bovine MDBK or murine L929 cells, as appropriate, using vesicular stomatitis virus as the challenge virus (18, 19). Human interferon titers are expressed in terms of the 69/19 or G023-901-527 reference standard for human-α interferon. Similarly murine interferon titers are expressed in terms of the G002-902-026 reference standard for murine interferon.

Neutralization of interferon by antisera.

Briefly, 50 µl aliquots of a 1 in 4 dilution of the oocyte incubation medium in Eagle's MEM containing 10% heat-inactivated fetal bovine serum were mixed with 2 µl of an appropriate antiserum, incubated at 37° for 60-90 minutes and the residual interferon titer estimated. Antisera to human interferon-α was obtained from Dr. E. A. Havell and from Dr. Karl Fantes, while that to human interferon-β was obtained from Dr. J. Vilček. The specificity of antisera to human interferon-α or β was verified

in separate experiments (not shown, also see Table 1 of ref. 11). Antisera to murine interferon-α (originally labeled anti-L-F) and to murine interferon-β (originally labeled anti-L-S) were a gift from Dr. Y. Kawade. The specificity of these antisera has been documented earlier (20).

Affinity chromatography of interferons through columns containing immobilized immunoglobulin to human interferon-β was carried out as described earlier for human interferon-α (21).

RESULTS

Interferon mRNA species in Sendai-induced human lymphoblastoid (Namalva) cells.

Fig. 1 illustrates the results obtained when polyadenylated RNA from Sendai-induced Namalva cells was electrophoresed through an agarose-CH_3HgOH tube gel. The mRNA eluted from adja-

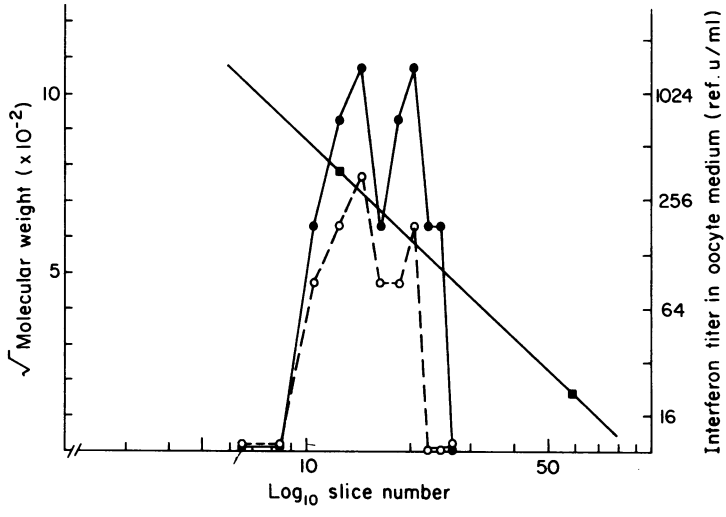

Figure 1. Gel electrophoresis of human interferon mRNA species from Sendai-induced Namalva cells. The activities of the oocyte translation products were assayed on human GM2504 (●) and bovine MDBK (○) cells. Approximately 250 μg polyadenylated RNA was denatured in 12.5 mM CH_3HgOH and electrophoresed through a 2% agarose-10 mM CH_3HgOH tube gel. Despite the amount of RNA loaded on the gel, the profile of ^{32}P-labeled marker rRNA and tRNA was well resolved. Each eluted mRNA sample was dissolved in 5 μl sterile water and a 2 μl aliquot was injected into ∿20 oocytes. Marker 18S and 4S RNA (■).

cent gel slices was injected into oocytes and 40 hours later the oocyte incubation medium harvested for assays on human GM2504 and on bovine MDBK cells. It is clear from Fig. 1 that the distribution of interferon mRNA species is heterogeneous with respect to the size of the molecules. At least two populations of interferon mRNA can be resolved by assay of the oocyte products on human cells. The profile of interferon activity on bovine MDBK cells indicates that there exist at least two species of human interferon-α mRNA in this preparation because human interferon-α (HuIFN-α) is known to show marked activity on bovine MDBK cells whereas human interferon-β (HuIFN-β) does not (22). Furthermore, since two of the samples towards the lower molecular weight side of the profile exhibit marked activity on human cells but no detectable activity on bovine cells it appears likely that there also exists a short mRNA for HuIFN-β in this preparation. Thus the data in Fig. 1 suggest that mRNA preparations from induced Namalva cells contain at least 3 mRNA species - two α and a short β.

The antigenic type of interferon synthesized by oocytes in response to mRNA eluted from gel slices was characterized by neutralization with monospecific antisera directed against HuIFN-α and HuIFN-β. Interferon activity that survives neutralization with anti-HuIFN-α is thus ascribed to β interferon and vice versa. Fig. 2 illustrates two separate experiments in which the oocyte products were assayed without antiserum admixture, after neutralization with anti-HuIFN-α alone or anti-HuIFN-β alone or both anti-HuIFN-α and anti-HuIFN-β. It is clear that at least two species each of HuIFN-α and HuIFN-β mRNA can be resolved. The most abundant mRNA is an α species of length approximately 1.3 kb, followed by a second α mRNA of length approximately 1.8-1.9 kb. The shoulder of activity on the low molecular weight side in Fig. 1, which was seen when interferon was assayed on human cells but was not seen on bovine cell assays can now be clearly attributed to a species of β mRNA of length approximately 1.1 kb (Fig. 2A & B). Furthermore a fourth interferon mRNA, that for HuIFN-β, is clearly detectable at approximately 1.9 kb (Fig. 2A & B). It should be noted that all interferon activity is completely neutralized by a mixture of anti-α

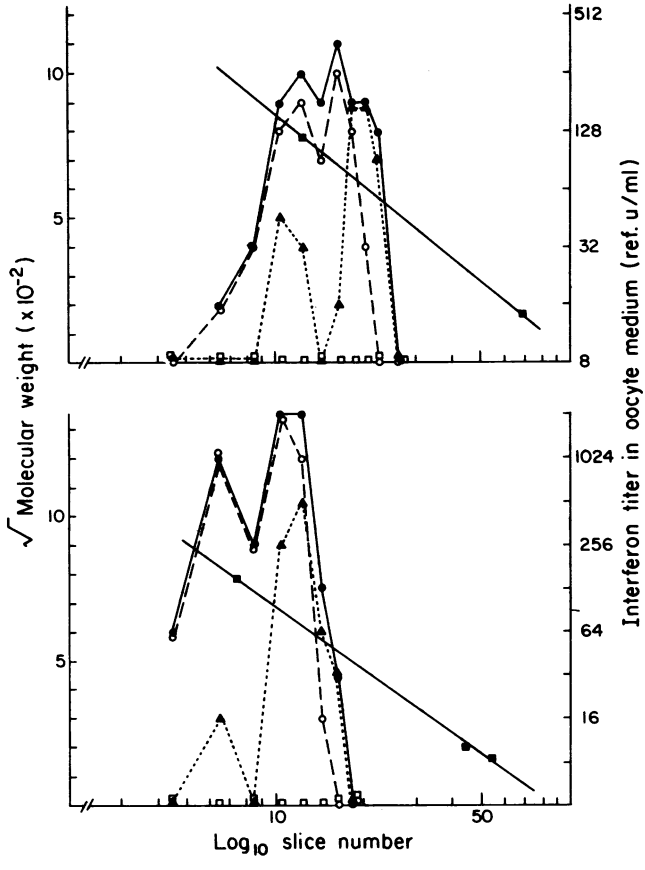

Figure 2. Neutralization of the oocyte translation products of human interferon mRNA species from Sendai-induced Namalva cells (●), by anti-HuIFN-α (▲) or anti-HuIFN-β (O) antisera alone or by both antisera (□). Residual interferon titers were assayed on human GM2504 cells. Marker 18S, 5S & 4S RNA (■).

Panel A. Neutralization of the translation products derived by microinjection into oocytes of the remaining 3 µl aliquot of each eluted mRNA sample described in Fig. 1.

Panel B. Approximately 75 µg of mRNA was electrophoresed as indicated in Fig. 1, each eluted mRNA sample dissolved in 5 µl water, a 2 µl aliquot microinjected into oocytes, and the antigenic nature of the oocyte translation products characterized.

and anti-β interferon antisera indicating the absence of any other kind of interferon mRNA in the preparation, eg. γ or immune interferon mRNA.

Since the two short HuIFN-α and HuIFN-β mRNA activities illustrated in Fig. 2A & B can be at least partially resolved by gel electrophoresis, they may be considered distinct interferon mRNA populations. The two long HuIFN-α and HuIFN-β mRNA activities in Fig. 2 could, however, represent a single population of interferon mRNA which gives rise to a protein with hybrid α and β interferon properties. That the α and β interferons synthesized from mRNA of length approximately 1.9 kb represent separate polypeptides was established by the observation that α interferon synthesized from the 1.9 kb mRNA fails to bind to an affinity chromatography column containing immobilized anti-HuIFN-β immunoglobulin (data not shown). Thus the β determinants in the translation product of the 1.9 kb mRNA reside on protein molecules that are physically distinct from the α determinants. All of the data taken together allow the conclusion that Sendai-induced Namalva cells contain at least 4 distinct interferon mRNA species.

Interferon mRNA species in NDV-induced murine (L) cells.

Fig. 3 illustrates that L-cell murine interferon (MuIFN) mRNA could be resolved into two size populations by electrophoresis through agarose-CH_3HgOH gels: an abundant population of length approximately 1.4 kb and a less abundant but reproducible population of length approximately 3 kb. The antiserum neutralization experiment presented in Table 1 shows that the translation products of each of these two populations of mRNA consist of both MuIFN-α and MuIFN-β. Thus both the 1.4 kb and 3.0 kb populations of murine interferon mRNA are likely to contain at least one species each of MuIFN-α and MuIFN-β mRNA.

DISCUSSION:

We have electrophoresed interferon mRNA preparations through agarose gels under stringent denaturing conditions in the presence of 10 mM CH_3HgOH. This technique has already helped resolve two distinct species of human fibroblast interferon mRNA (labeled β_1 and β_2) present in poly(I)·poly(C)-in-

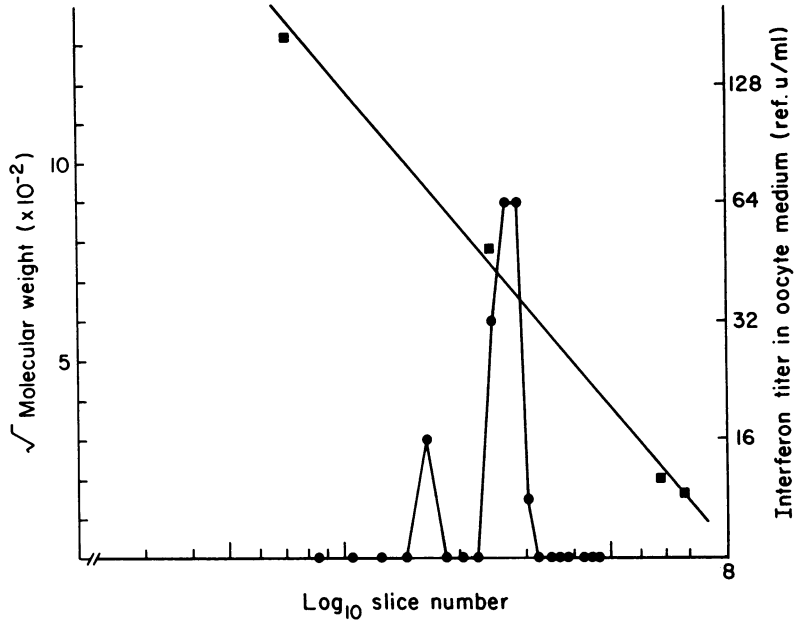

Figure 3. Gel electrophoresis of murine interferon mRNA species from NDV-induced Lpa cells. Approximately 40 µg of polyadenylated RNA from induced L cells was electrophoresed through a 2% agarose-10 mM CH_3HgOH tube gel and the interferon activity synthesized by oocytes in response to half of each mRNA fraction eluted from gel slices was assayed twice on L929 cells (●). The figure illustrates the geometric mean interferon titers observed. Marker 28S, 18S, 5S and 4S RNA (■).

duced human diploid cells (FS-4) (11,12) [In accordance with current guidelines for interferon nomenclature, designation of interferon subtypes using arabic numeral subscripts indicates that sufficient amino-acid or nucleotide sequence information is available to support a distinction between the subtypes]. This electrophoretic procedure combined with neutralization of oocyte translation proudcts with human or murine interferons-α or β specific antisera has now allowed us to detect at least four species of human interferon mRNA in virus-induced Namalva cells and has indicated the presence of four species of murine interferon mRNA in virus-induced L cells. Thus, these procedures provide an insight into the remarkable heterogeneity of

Table 1. Neutralization of murine interferons synthesized by Xenopus oocytes in response to microinjection with separated 3 kb and 1.4 kb mRNA species.

MuIFN mRNA injected	Interferon titer (ref. U/ml)			
	No antiserum	Anti-MuIFN-α	Anti-MuIFN-β	Anti-MuIFN α and β
3 kb	256	128	256	≤24
	362	96	96	≤16
1.4 kb	1024	512	1024	≤16
	1024	512	384	≤16

60 µg cytoplastmic polyadenylated RNA from NDV-induced L cells was electrophoresed through a 2% agarose-10 mM CH_3HgOH tube gel, the eluted mRNA dissolved in 5 µl water and the interferon mRNA species located as described in Fig. 3. Two fractions each of mRNA approximately 3 kb and 1.4 kb in length were microinjected into ∿20 oocytes (2 µl RNA) and the murine interferon secreted into the incubation medium was characterized by neutralization with anti-MuIFN-α and anti-MuIFN-β antisera followed by assay of the residual interferon activity on L929 cells.

translatable human and murine mRNA species for interferons of both the α and β type. Furthermore, since the recovery of translatable mRNA from agarose-CH_3HgOH gels is in the range 60-80%, this electrophoretic technique provides an important preparative purification procedure for the molecular cloning of the numerous interferon mRNA species that have now been observed.

Approximately 70% of the interferon produced by virus-induced Namalva cells is of the α type while the remainder is of the β type (23). The HuIFN-α from these cells has been shown to consist of at least 5 different polypeptides in the range 18,500 to 21,500 daltons (24). The HuIFN-β component has not yet been characterized. Cavalieri et al. (25) have shown that polyadenylated mRNA obtained from virus-induced Namalva cells leads to the synthesis in Xenopus oocytes of interferon which is approximately 70% α- and 30% β-type. Furthermore, Morser et al. (7) and Berger et al. (8) have shown that the predominant interferon mRNA species in such preparations sediments at approximately 12-13S in sucrose gradients. In addition Berger et al. (8) report the detection of interferon mRNA in Namalva

cells which sediments at approximately 18S in sucrose gradients. The data presented in Figs. 1 & 2 are consistent with the earlier observations. In addition Figs. 1 & 2 reveal a remarkable degree of heterogeneity in interferon mRNA populations from induced Namalva cells. Though the distribution of HuIFN-α mRNA in agarose-gels displays two peaks of activity it is clearly more heterogeneous than that of the two HuIFN-β species. Thus it is likely that there exist more than 2 species of HuIFN-α in mRNA preparations from Namalva cells.

The protein counterparts of HuIFN-β (1.1 kb) and HuIFN-β (1.9 kb) are as yet unknown. These two β mRNA species differ in length from HuIFN-β_1 and HuIFN-β_2 isolated from poly(I).poly(C)-induced diploid fibroblasts (FS-4). The mRNA for HuIFN-β_1 has a length of 0.9 kb while that for HuIFN-β_2 has a length of 1.3 kb in agarose-CH_3HgOH gels (11,12). Molecular cloning and nucleic hybridization analyses are in progress in order to determine the relationship between the various HuIFN-β mRNA species.

Virus-induced murine L cells produce at least two species of interferon proteins as judged by electrophoresis on SDS-polyacrylamide gels: MuIFN-α with an apparent molecular weight in the range 22,000-24,000 daltons and MuIFN-β with an apparent molecular weight in the range 35,000-40,000 daltons (26-29). However, Erickson and Paucker (30) have used a combination of affinity chromatography on hydrophobic ligands and SDS-polyacrylamide gel electrophoresis to resolve a total of five molecular species of NDV-induced L cell interferon. The data in Fig. 3 and Table 1 taken together suggest the presence of at least 4 interferon mRNA species in polyadenylated RNA from induced L cells, two each for MuIFN-α and MuIFN-β. Molecular cloning of the cDNAs corresponding to these interferon mRNA species should help eluciddate the relationships among murine interferon genes and between the murine and human interferon genes.

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