Proc. Natl. Acad. Sci. USA
Vol. 78, No. 3, pp. 1741–1745, March 1981
Cell Biology

Translational activity and functional stability of human fibroblast β_1 and β_2 interferon mRNAs lacking 3′-terminal RNA sequences

[FS-4 cells/poly(I)·poly(C)/polynucleotide phosphorylase (polyribonucleotide nucleotidyltransferase, polyribonucleotide:orthophosphate nucleotidyltransferase)/rabbit reticulocyte lysate/*Xenopus laevis* oocytes]

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Communicated by Igor Tamm, December 5, 1980

ABSTRACT **Polyadenylylated mRNA was purified from poly(I)·poly(C)- and cycloheximide-superinduced human fibroblast (FS-4) cultures. The mRNA was subjected to electrophresis through an agarose/CH_3HgOH gel, and human fibroblast β_1 and β_2 interferon mRNAs were isolated. Each mRNA preparation was phosphorolyzed at 0°C for 20 min by using a molar excess of polynucleotide phosphorylase to produce RNAs lacking poly(A) and then incubated at 37°C for varying lengths of time to allow the phosphorylase to further digest the deadenylylated RNA from the 3′ end in a processive and synchronous manner. Removal of the poly(A) (≤100 residues) and ≈100 adjacent residues from human fibroblast β_1 interferon mRNA (native length, 900 residues, including a 3′-noncoding region of 203 residues) did not alter the translational activity or the functional stability of this mRNA in *Xenopus* oocytes, whereas deletion of the poly(A) and ≈200 adjacent residues decreased its translational efficiency. On the other hand, removal of the poly(A) (≈200 residues) and ≈200 adjacent residues from human fibroblast β_2 interferon mRNA (native length, 1300 residues) did not alter the translational activity or the functional stability of this molecule in oocytes. Thus, neither the poly(A) nor large segments of the 3′-noncoding region (which includes the hexanucleotide A-A-U-A-A-A sequence, at least in the case of β_1 mRNA) are required for the maintenance of the functional stability of human β_1 and β_2 interferon mRNAs in *Xenopus* oocytes.**

Mammalian mRNAs have an extensive 3′-terminal noncoding region that lies between the termination codon for the translation product and the poly(A) site or, in the case of poly(A)-free mRNA, the 3′ end of the RNA molecule. The 3′-noncoding regions of mammalian mRNAs contain areas of striking RNA homology, such as the A-A-U-A-A-A hexanucleotide sequence (1). Numerous investigators have studied the role of the poly(A) segment in the translational efficiency and the functional and physical stability of various mammalian and viral mRNA species (2–10). Enzymatic deadenylylation decreases the functional and physical stability of rabbit globin mRNA in *Xenopus laevis* oocytes (2–6), and the poly(A)-free human (HeLa) histone mRNA species can be stabilized by enzymatic adenylylation (7). Enzymatic deadenylylation, however, does not appear to decrease the functional stability of human fibroblast interferon mRNA microinjected into *Xenopus* oocytes (8). The functional stabilities of mengovirus RNA (9) and of mRNA 4 from alfalfa mosaic virus (11) in *Xenopus* oocytes are also not affected by the presence or absence of 3′ poly(A) tails.

The role of 3′-noncoding RNA regions in the translational efficiency of mammalian mRNA species has also been investigated (9, 12–15). For example, rabbit globin mRNA from which almost the entire 3′-noncoding region has been enzymatically removed retains translational activity in wheat germ and reticulocyte lysate cell-free translation systems (12, 13). However, the effect of deleting the 3′-noncoding region on the functional stability of mRNA species remains unexplored.

We have used polynucleotide phosphorylase (PNPase; polyribonucleotide nucleotidyltransferase, polyribonucleotide:orthophosphate nucleotidyltransferase, EC 2.7.7.8) to prepare human fibroblast β_1 and β_2 interferon mRNA species lacking both the poly(A) and up to 200 residues of the 3′-terminal noncoding region adjacent to the poly(A). The translational efficiency and functional stability of the truncated mRNA molecules was investigated after microinjection into the oocytes of *Xenopus laevis*. In an earlier communication, we presented evidence suggesting that the functional stability of human fibroblast interferon mRNA preparations that had been enzymatically deadenylylated by PNPase and microinjected into *Xenopus* oocytes is indistinguishable from that of native polyadenylylated interferon mRNA (8). The discovery that such interferon mRNA preparations contain two distinct human fibroblast interferon mRNA species, designated β_1 and β_2 (16–18), has led us to consider the role of the poly(A) segment and to determine the involvement of the 3′-noncoding sequence in their functional stabilities.

MATERIALS AND METHODS

The procedures used have been described (2, 6, 15, 16, 19–26). Interferon titers (assayed on GM 2504 cells) are expressed in terms of the 69/19 reference standard for human interferon. Partially purified human fibroblast interferon (10^6 reference units per ml; 10^6 reference units per mg of protein) was a gift from I. Braude. [^{3}H]Uridine (26 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), [^{32}P]orthophosphate (carrier-free), [^{3}H]leucine (110 Ci/mmol), and [^{35}S]methionine (730 Ci/mmol) were purchased from New England Nuclear. Laboratory-bred *Xenopus laevis* were purchased from NASCO, Fort Atkinson, Wisconsin.

PNPase, purified from *Escherichia coli* by the procedure of Soreq and Littauer (27, 28) was used to deadenylylate mRNA at 0°C for 20 min [100 μl of reaction contained 80 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/20 mM sodium phosphate, pH 8.0/1 mM EDTA/up to 40 μg of mRNA and a molar excess of PNPase (10 μg of enzyme for 5–10 μg of mRNA)]. The reaction mixture was warmed to 37°C in a water bath and incubated for varying lengths of time, and the reaction was then stopped by the addition of 2 μl of 20% $NaDodSO_4$. The mixture was extracted with phenol/chloroform/isoamyl alcohol, and the RNA was precipitated with ethanol. The progress of the reaction was usually followed by monitoring the loss of Cl_3CCOOH-precipitable ^{3}H radioactivity during the phosphorolysis of [^{3}H]uridine-

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Abbreviations: PNPase, polynucleotide phosphorylase; TLC, thin-layer chromatography; kb, kilobase(s).

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labeled mRNA (28). Procedures for thin-layer chromatography (TLC) of the digestion products have been described (29).

RESULTS

Phosphorolysis of Polyadenylylated FS-4 Cell mRNA. [^{3}H]Uridine-labeled polyadenylylated cellular RNA was prepared from poly(I)·poly(C)-induced FS-4 cultures and digested with a molar excess of PNPase for 20 min at 0°C. This procedure completely removes the poly(A) sequences from all mRNA molecules in the reaction mixture (2, 9), giving a homogeneous population of mRNA chains ending at the junction between the 3′-noncoding region and the poly(A). That all of the poly(A) was removed by this procedure was confirmed by the observations that (*i*) ≥95% of the interferon mRNA activity in the digested samples failed to bind to a poly(U)–Sepharose column in 0.4 M NaCl at room temperature and therefore had a poly(A) tail of ≤30 residues (table 2 in ref. 8; refs. 30 and 31) and (*ii*) ≥95% of the [^{3}H]uridine radioactivity failed to bind to an oligo(dT)–cellulose column in 0.5 M NaCl at 4°C and therefore had a poly(A) tail of ≤12 residues (ref. 6; data not shown). Deadenylylated RNA that failed to bind to an oligo(dT)–cellulose column at 4°C was re-equilibrated with a molar excess of PNPase for 20 min at 0°C and then warmed to 37°C in a water bath. Phosphorolysis was stopped after 30, 60, 90, and 120 sec of incubation at 37°C by the addition of $NaDodSO_4$. Aliquots of the phenol-extracted reaction mixture were either subjected to TLC through Polygram CEL 300 polyethylenimine (Machery-Nagel) plates or were precipitated with Cl_3CCOOH. On the TLC plates, radioactivity was quantitated at the origin (undigested RNA) and in regions corresponding to UMP and UDP markers. Virtually all of the soluble radioactivity was found as UDP (data not shown), indicating the absence of endonucleolytic cleavage that would have produced [^{3}H]UMP (27–29). The extent of phosphorolysis measured by TLC or by loss of Cl_3CCOOH-precipitable radioactivity was similar (data not shown). Furthermore, the rate of phosphorolysis of polyadenylylated mRNA that had been incubated with PNPase for 20 min at 0°C and then warmed to 37°C was indistinguishable from that of deadenylylated mRNA that had been extracted with phenol and reselected by passage through an oligo(dT)–cellulose column at 4°C (data not shown). Thus, in all subsequent experiments, we digested the polyadenylylated mRNA at 0°C for 20 min and then warmed the reaction mixture to 37°C, monitoring the course of phosphorolysis by measuring the loss of Cl_3CCOOH-precipitable radioactivity. The average number of nucleotides trimmed per RNA chain was estimated by relating the fractional loss of Cl_3CCOOH-precipitable radioactivity to the median length of the RNA chains in the preparation (unfractionated RNA has 1600 residues; sucrose gradient-selected 11–14S mRNA has 1150 residues).

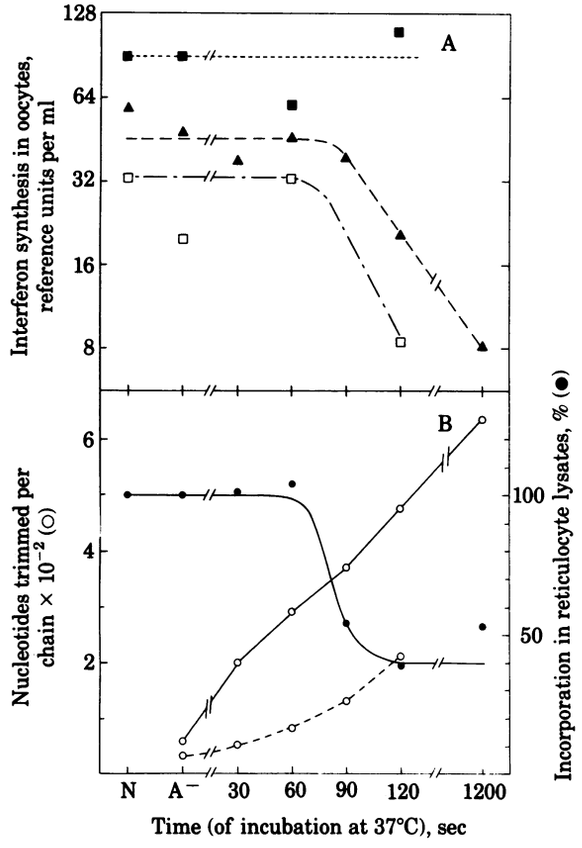

FIG. 1. Translational efficiency of phosphorolyzed cellular (FS-4) and interferon mRNAs. (*A*) Survival of interferon mRNA activity, as assayed in *Xenopus* oocytes, after phosphorolysis of unfractionated interferon mRNA preparations (▲) or of agarose/CH_3HgOH gel-purified β_1 (□) or β_2 (■) interferon mRNA species. (*B*) Survival of cellular mRNA activity, as assayed in reticulocyte lysates, after phosphorolysis of [^{3}H]uridine-labeled polyadenylylated RNA preparations from induced FS-4 cells (●) and average number of nucleotides trimmed per RNA chain from unfractionated RNA (○——○) or sucrose gradient-selected 11–14S RNA (○— —○). Polyadenylylated RNA was prepared by poly(U)/Sepharose chromatography of RNA from FS-4 cells grown in 150-mm Falcon Petri dishes induced with poly(I)·poly(C) (P-L Biochemicals, 30 μg/ml) in 10 ml of Eagle's minimal essential medium/cycloheximide (Calbiochem, 50 μg/ml) for 4 hr and labeled with [^{3}H]uridine (10 μCi/ml) during the induction period. Poly(A)-containing RNA (10–20 μg for each time point) was either directly phosphorolyzed or was first fractionated through a sucrose gradient or an agarose/CH_3HgOH gel and then phosphorylyzed. Suitable aliquots of 3′-trimmed mRNA (approximately half of each sample) were translated in a rabbit-reticulocyte lysate system (NEN) and the incorporation of either [^{3}H]leucine or [^{35}S]methionine was monitored (100% levels represent 10,590 cpm/20 μl of reaction mixture for [^{3}H]leucine incorporation or 15,690 cpm/10 μl of reaction mixture for [^{35}S]methionine above background incorporation). The remaining half of each RNA sample was microinjected into groups of 15–20 *Xenopus* oocytes. N, native mRNA; A⁻, poly (A)-free mRNA.

Phosphorolysis of deadenylylated total FS-4 cell mRNA by PNPase at 37°C proceeded in a linear manner for up to 120 sec, leading to the removal of ≈500 residues per RNA chain (Fig. 1B). However, digestion of sucrose gradient-selected 11–14 mRNA was slower. Approximately 200 residues adjacent to the poly(A) were removed by digestion for 120 sec. This agrees with the observation that PNPase phosphorolyzes individual RNA species at different rates (for review, see ref. 9). Furthermore, the rate of digestion of sucrose gradient-selected 11–14S mRNA correlated well with the reduction in length of interferon mRNA species, as monitored by electrophoresis in agarose/CH_3HgOH gels (Table 1).

Table 1. Average lengths of PNPase-digested human fibroblast interferon mRNAs, as estimated by electrophoresis in agarose/CH_3HgOH gels

Digestion conditions	Length, kb β_1	Length, kb β_2
Native (no PNPase)*†	0.9	1.3
PNPase at 4°C for 20 min‡	0.8	1.1
PNPase at 4°C for 20 min and then 37°C for 60 sec‡	0.7	1.0

Electrophoretic analyses were similar to those shown in Fig. 2.
* SEM ≈40 residues.
† $n = 6$.
‡ $n = 3$.

Translational Activity of Phosphorolyzed FS-4 Cell mRNA. Removal of the poly(A) and further digestion of FS-4 cell mRNA preparations with PNPase at 37°C for up to 60 sec did not alter the translational activity of the RNA when assayed in rabbit reticulocyte lysates (see Fig. 1*B*). However, digestion for 120 sec decreased this activity to 40% of the native preparation, although prolonged incubation (20 min) did not decrease it any further.

Translational Activity of Phosphorolyzed Human Fibroblast β_1 and β_2 Interferon mRNA. Polyadenylylated mRNA preparations from poly(I)·poly(C)-induced FS-4 cell cultures contain two distinct species of human fibroblast interferon mRNA (16, 18). The β_1 mRNA is 0.9 kilobases (kb) long and the β_2 mRNA is 1.3 kb long. The 3′-noncoding region of β_1 mRNA is 203 residues long and includes an A-A-U-A-A-A sequence at a distance of 20 residues from the poly(A) (17). In contrast, the 3′-noncoding region of β_2 mRNA may be 438 residues long and includes two A-A-U-A-A-A hexanucleotides at distances of 72 and 9 residues from the poly(A) (18, M. Revel, personal communication).

Interferon mRNA activity of total mRNA preparations from induced FS-4 cells, assayed in *Xenopus* oocytes (interferon synthesis was measured during the first 24–36 hr), was unaffected by removal of the poly(A) and by digestion for a further 60–90 sec at 37°C (see Fig. 1*A*). Digestion for 120 sec or for 20 min decreased interferon mRNA activity. Similarly, removal of the poly(A) and digestion for 60 sec at 37°C did not significantly alter the ability of gel-purified β_1 interferon mRNA to direct the synthesis of interferon, although digestion for 120 sec decreased this ability. In contrast, β_2 interferon mRNA retained its ability to direct interferon synthesis even after digestion for up to 120 sec.

Digestion by PNPase of globin mRNA, artemia mRNA, carnation mottle virus RNA, and tobacco mosaic virus RNA proceeds with a synchrony of ±10 residues (9, 14, 15, 28). Synchrony of PNPase digestion of β_1 and β_2 interferon mRNA species was verified by electrophoresis of 3′-trimmed mRNA samples through agarose/CH_3HgOH tube gels (16). Phosphorolyzed β_1 and β_2 interferon mRNA species were resolved sharply with little or no widening of the activity peaks after deadenylylation and after further digestion for 60 sec at 37°C (Fig. 2). Furthermore, Fig. 2*C* shows that β_1 mRNA ≈0.65 kb long and β_2 mRNA ≈0.85 kb long were translationally active mRNA molecules (the error in these length measurements is ≈50 residues; in this gel, in the 0.6- to 0.9-kb region, adjacent gel slices correspond to this difference in RNA length). The *average* rates of digestion of β_1 and β_2 interferon mRNA, as determined by gel electrophoresis, correlated well with the rate of solubilization of ^3H radioactivity from [^3H]uridine-labeled, sucrose gradient-selected mRNA (see Fig. 1*B*, which shows that ≈100 residues were deleted in 60 sec, and Table 1). The gel electrophoretic analyses also suggest that β_1 interferon mRNA has a shorter poly(A) (≤100 residues) than β_2 interferon mRNA (≈200 residues). The length of deadenylylated β_1 interferon mRNA, as estimated by gel electrophoresis (0.8 kb), is in good agreement with length estimates based on recent sequencing data (836 residues; refs. 17 and 32).

Thus, our data suggest that removal of the poly(A) and ≈100 residues from the 3′-noncoding region of β_1 interferon mRNA or ≈200 residues from that of β_2 interferon mRNA does not decrease the translational activity of these mRNA molecules in *Xenopus* oocytes, even though the deleted regions include the hexanucleotide A-A-U-A-A-A. On the other hand, removal of ≈200 residues from β_1 mRNA does decrease its translational

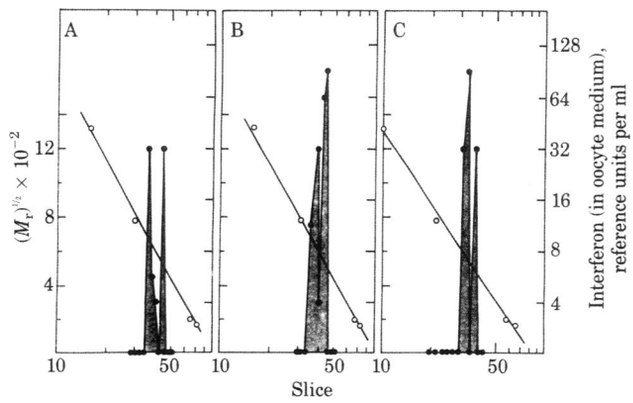

FIG. 2. Agarose (2%)/CH_3HgOH (10 mM) gel electrophoresis of native and phosphorolyzed interferon mRNAs. (*A*) Native. (*B*) Treated with PNPase for 20 min at 4°C. (*C*) Treated with PNPase for 20 min at 4°C and then for 60 sec at 37°C. Approximately 40 μg of poly(A)-containing RNA from induced FS-4 cells was phosphorolyzed, reextracted, and subjected to electrophoresis on 0.6 × 11 cm tube gels as described (16). RNA length markers (○) represent ^{32}P-labeled HeLa 28S, 18S, 5S, and 4S RNA that was admixed with the interferon mRNA samples before electrophoresis. Interferon mRNA activity (●) was assayed in *Xenopus* oocytes in RNA eluted from pools of adjacent 1-mm-thick gel slices.

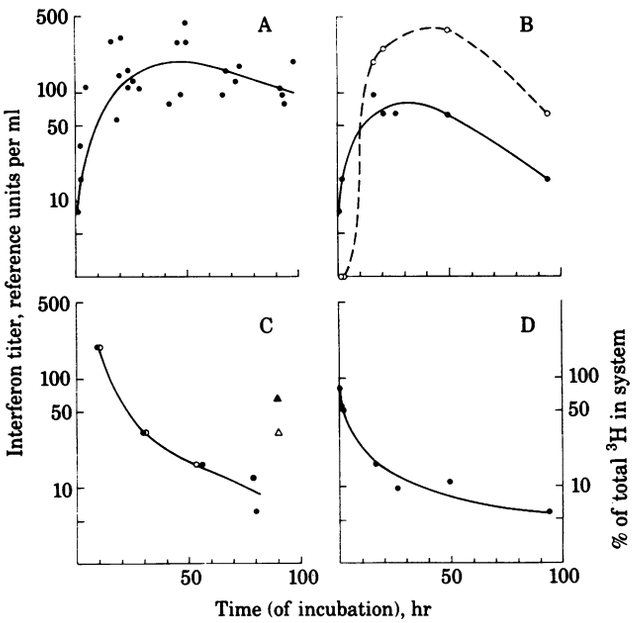

FIG. 3. Kinetics of interferon synthesis by *Xenopus* oocytes. Groups of oocytes were microinjected with [^3H]uridine-labeled polyadenylylated RNA from induced FS-4 cells (≈15 oocytes per group; ≈100 ng of RNA in 80–100 nl of sterile water per oocyte) and incubated in 0.2 ml of modified Barth's medium (33) at 22–25°C. At different intervals thereafter, the interferon contents of the incubation medium and of the oocytes (homogenized in a fresh 0.2-ml aliquot of Barth's medium) were determined. (*A*) Total cumulative yield (sum of interferon titers in the incubation medium and inside the oocytes); pooled data of four experiments. (*B*) Interferon content inside the oocytes (●) and in the incubation medium (○) in a typical experiment. (*C*) Daily export of interferon from oocytes. Two separate groups of oocytes (≈20 each) were injected with RNA solution [20 nl per oocyte (30 ng per oocyte)], and the daily interferon export was monitored by 20–24 hourly changes of medium (● and ○). Approximately 90 hr after microinjection, the oocytes were homogenized in 0.2 ml of fresh medium, and the interferon content was determined (▲ and △). (*D*) Survival of Cl_3CCOOH-precipitable [^3H]uridine-labeled mRNA inside the oocytes in the experiment shown in *B* and also included in *A*. The 100% level corresponds to 24,535 cpm per 10 oocytes.

Table 2. Fate of human fibroblast interferon microinjected into *Xenopus* oocytes

	Interferon titer, reference units per ml			
	15 min after microinjection		20 hr after microinjection	
Experiment	Medium	Oocytes	Medium	Oocytes
1	32	512	32	8
2	16	1024	16	8
	≤6	1536	≤6	12
3	6	192	16	≤4
	≤4	192	≤4	≤4

In experiments 1 and 2, 80–100 nl of interferon solution (10^6 reference units per ml) was injected per oocyte, and in experiment 3, 50 nl was injected per oocyte. Groups of 10 oocytes were incubated in modified Barth's medium, and the interferon contents in the medium and inside the oocytes (homogenized in 0.2 ml of fresh medium) were determined.

activity. This corresponds to a loss of almost the entire 3'-noncoding region of the β_1 molecule.

Functional Stability of Native Human Fibroblast Interferon mRNA. The total cumulative yields of interferon from oocytes incubated for varying lengths of time are shown in Fig. 3*A*. These observations reproduce our earlier results (8, 19) and correlate well with similar observations of other investigators (34–36). Interferon is detectable within 1 hr of microinjection of its mRNA into oocytes, and the total cumulative yield appears to peak in ≈48 hr. Similar results (not shown) were obtained when only 20 nl of RNA solution was injected into each oocyte or when the oocytes were incubated at 18°C or in a larger volume of Barth's medium (20 oocytes in 1 ml).

Because interferon is rapidly exported from the intracellular oocyte compartment into the incubation medium (ref. 37 and Fig. 3*B*), we also monitored this export over a 4-day period. Groups of oocytes (≈20 per group) were injected with interferon mRNA solutions (20 nl per oocyte) and incubated in 0.2 ml of modified Barth's medium, and the rate of export of interferon was followed by daily changes of incubation medium (see Fig. 3*C*). The apparent rate of export of interferon decayed in a biphasic manner: an initial phase having $t_{1/2}$ 6–10 hr that accounted for most of the decay and a late phase having $t_{1/2}$ ≈30 hr. Similar results were obtained when oocytes were incubated in a larger (1 ml) volume of medium or when 90–100 nl of mRNA solution was injected into each oocyte (data not shown). The biphasic decay of interferon export correlated well with the biphasic decay of Cl_3CCOOH-precipitable [^{3}H]uridine-labeled native FS-4 cell mRNA preparations injected into oocytes (see Fig. 3*D*) which, in turn, correlated well with previous observations describing the survival of microinjected RNA in *Xenopus* oocytes (38–40).

Table 3. Synthesis of interferon in oocytes microinjected with interferon mRNA a second time

	Microinjection		Interferon titer (at 72 hr), reference units per ml		
Group	0 hr	48 hr later	Medium	Oocytes	Total
1A	+	−	≤4	90	90
1B	+	+	76	304	380
1C	−	+	181	107	288
2A	+	−	≤4	32	32
2B	+	+	23	90	113
2C	−	+	16	32	48

Groups of ≈15 oocytes were microinjected with interferon mRNA (groups 1A and 1B and 2A and 2B; 50 ng of mRNA in ≈80 nl of water per oocyte) and incubated in 0.2 ml of Barth's medium for 48 hr. Controls (groups 1C and 2C) were incubated without microinjection. At 48 hr, the oocytes were rinsed and appropriate groups (1B and 1C and 2B and 2C) were injected with interferon mRNA (15 ng of mRNA in ≈70 nl of water per oocyte). All oocytes were then incubated in 0.2 ml of fresh Barth's medium for another 24 hr. Interferon titers in the incubation medium at 48 hr for groups 1A, 1B, 2A, and 2B were 64, 128, 48, and 32 reference units per ml, respectively.

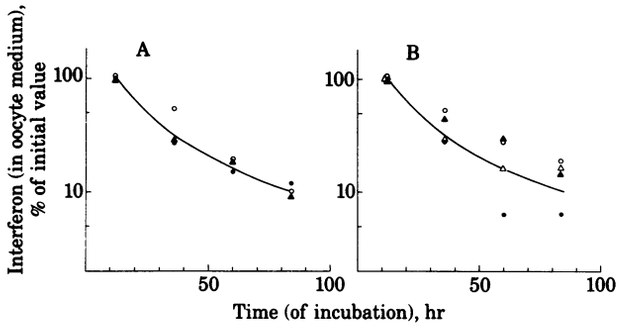

FIG. 4. Functional stabilities of native and phosphorolyzed human fibroblast β_1 and β_2 interferon mRNAs. Human β_1 and β_2 interferon mRNAs were isolated from agarose/CH_3HgOH tube gels (representing the equivalent of 65 μg of FS-4 cell mRNA), reselected on oligo(dT)–cellulose, and phosphorolyzed as described in the legend to Fig. 1; each sample was microinjected into groups of ≈20 oocytes (≈80 nl per oocyte), and the daily export of interferon was monitored as described in the legend to Fig. 3*C*. (*A*) β_1 mRNA. (*B*) β_2 mRNA. ●, Native; ○, deadenylylated; △, treated with PNPase at 37°C for 60 sec; ▲, treated with PNPase at 37°C for 120 sec. One hundred percent levels are given in Fig. 1 and correspond to 33 and 96 reference units per ml for *A* and *B*, respectively.

Because our kinetic data describing interferon synthesis by *Xenopus* oocytes (refs. 8, 19 and Fig. 3) differ from those of Colman and Morser (37), who describe interferon synthesis as persisting at high levels for several days, we carried out two additional kinds of control experiments. We found that partially purified human fibroblast interferon microinjected into the cytoplasmic compartment of the oocyte does not leak into the incubation medium but decays rapidly within the oocyte (Table 2). These observations agree with those of Colman and Morser (37), who have reported the rapid decay of human lymphoblastoid interferon activity injected into *Xenopus* oocytes. We also found that oocytes that were injected with interferon mRNA 48 hr after a previous injection showed a second burst of interferon synthesis (Table 3). Thus, the decay of interferon synthesis that we observed (see Fig. 3*C*) does not reflect damage to the translational apparatus of microinjected oocytes.

Functional Stability of Phosphorolyzed Human Fibroblast β_1 and β_2 Interferon mRNA. The functional stability of phosphorolyzed β_1 interferon mRNA was assayed by following the daily export of interferon (see Fig. 3*C*) from groups of oocytes that had been microinjected with native, deadenylylated or 3'-trimmed (100 residues removed) human β_1 interferon mRNA preparations (Fig. 4*A*). Similar results were obtained when native, deadenylylated and 3'-trimmed (100 or 200 residues removed) human β_2 interferon mRNA preparations were microinjected into oocytes (Fig. 4*B*). Thus, the functional stabilities of both native β_1 and β_2 interferon mRNA species are indistinguishable from each other and from those of their deadenylylated and 3'-trimmed counterparts.

DISCUSSION

We have investigated the translational activity of human fibroblast β_1 and β_2 interferon mRNAs lacking the poly(A) segment and up to ≈200 residues of the 3'-noncoding region in a het-

erologous *in vivo* translation system, the *Xenopus* oocyte. We observed that removal of the poly(A) and up to 100 residues of β_1 interferon mRNA and up to 200 residues of β_2 interferon mRNA does not detectably alter their translational activity. However, deletion of the poly(A) and ≈200 adjacent residues, which make up almost the entire 3'-noncoding region of β_1 mRNA, decreases its translational activity. Deletion of the poly(A) and up to 100 adjacent residues from β_1 mRNA or up to 200 adjacent residues from β_2 mRNA does not alter the functional stability of these mRNAs, as monitored over a 4-day period after microinjection into oocytes.

It is believed that the presence of a 3' poly(A) tail helps stabilize mammalian mRNAs. This belief arises mainly from the convincing demonstration that removal of the poly(A) from rabbit globin mRNA preparations greatly reduces the physical and functional stability of the molecule in *Xenopus* oocytes (2–6). The observation that adenylylation of human (HeLa) histone mRNAs increases their functional stability in oocytes corroborates these findings (7). However, data obtained by using interferon mRNA (8), mengovirus mRNA (9), and mRNA 4 from alfalfa mosaic virus (11) suggest that the stabilizing role of poly(A) is not applicable to all mRNAs microinjected into oocytes. Indeed, Woodland and Wilt (40) report that a small fraction of poly(A)-free sea urchin histone mRNA can be functionally stable for several weeks in *Xenopus* oocytes. It also appears that deletion of large segments of the 3'-noncoding region, including the A-A-U-A-A-A hexanucleotide, does not affect the translational efficiency (refs. 9, 12, and 13, and Fig. 1) or the functional stability (see Fig. 4) of deproteinized mammalian mRNAs introduced into a heterologous translation system—i.e., the *Xenopus* oocyte.

We thank Drs. Igor Tamm, Ricardo Cortese, Elaine Diacumakos, U. Z. Littauer, and Michel Revel for helpful discussions and Mr. Ira Augenzucker and Mrs. Rivka Zisling for excellent technical assistance. This investigation was supported by Grant AI-16262 from the National Institute of Allergy and Infectious Diseases and by the Israeli Commission for Basic Research. H.S. is the recipient of a Charles Revson Career Development Award, A.D.S. is supported by an Institutional Training Grant from the National Cancer Institute, and P.B.S. is the recipient of a Junior Faculty Research Award from the American Cancer Society.

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