

Interferon messenger RNA content of human fibroblasts during induction, shutoff, and superinduction of interferon production

[human diploid fibroblast cells/poly(I)·poly(C)/McAuslan–Tomkins translational repressor hypothesis/5,6-dichloro-1- β -D-ribofuranosylbenzimidazole/*Xenopus laevis* oocytes]

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ABSTRACT Translation of injected mRNA in oocytes of *Xenopus laevis* has been used as a highly sensitive and quantitative assay for interferon mRNA. Injection into oocytes of polyadenylated RNA extracted from poly(I)·poly(C)-induced human diploid fibroblasts (FS-4) leads to the synthesis of biologically active human fibroblast interferon over a period of 24–32 hr. There is a linear relationship between the amount of mRNA injected and the interferon yield obtained over a range of 1–20 ng of injected RNA. Injection of 40–80 ng of mRNA into each of 15 oocytes, homogenized in 0.3 ml of incubation medium, gave a titer of 128–256 interferon reference units/ml of homogenate.

FS-4 cells at the peak of interferon production—i.e., approximately 2.5 hr after the beginning of induction with poly(I)·poly(C)—gave mRNA that yielded 24–48 interferon reference units/ml in the oocyte assay (30 ng of RNA injected per oocyte). An equivalent amount of mRNA from FS-4 cells in the shutoff phase, approximately 6 hr after induction, gave ≤ 4 interferon reference units/ml. In contrast, mRNA extracted from FS-4 cells that had been induced and maintained in the presence of 40 μ M 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole for 6 hr produced 64–128 interferon reference units/ml. Polyadenylated RNA obtained from uninduced FS-4 cells did not lead to detectable interferon synthesis (< 4 interferon reference units/ml). These data provide a direct verification of the hypothesis that the shutoff of interferon production in FS-4 cells involves a regulatory event leading to the posttranscriptional inactivation or degradation of interferon mRNA. Because the inactivating mechanism is sensitive to inhibition by 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole, a selective inhibitor of nuclear heterogeneous RNA and mRNA synthesis, it is likely that synthesis of an RNA molecule is necessary for the shutoff of interferon production.

Human fibroblast interferon is a glycoprotein with an apparent molecular weight of 21,000 (1, 2) and is characterized by the F antigenic determinant (3). Cultures of human diploid fibroblasts (FS-4 strain) can be induced to synthesize and secrete interferon into the extracellular medium by exposure to poly(inosinic acid)·poly(cytidylic acid) [poly(I)·poly(C)] (4–8). Extensive studies with inhibitors of RNA or protein synthesis indicate that the regulation of interferon production in poly(I)·poly(C)-induced FS-4 cells is complex (7, 9–11). Fig. 1 presents a summary of the key events thought to underlie poly(I)·poly(C)-induced interferon production in FS-4 cells, as revealed through inhibitor studies (10).

Interferon production in FS-4 cells reaches detectable levels approximately 1 hr after the beginning of induction, rises to a peak by 2.5–3 hr, and is rapidly shut off by 6–8 hr (5, 10, 12). Inhibitors of RNA or protein synthesis, applied before the shutoff, prevent the shutoff and lead to a continuous production of interferon for up to 4 days (7, 9, 10, 12, 13). As a result, there occurs a paradoxical increase (“superinduction”) of up to a 100-fold in the cumulative interferon yield (7, 9, 13–15). Detailed analyses of the kinetics of interferon production by poly(I)·poly(C)-induced FS-4 cells have led to the suggestion that the rate of interferon production in response to poly(I)·

poly(C) is determined by two processes (7, 16). The inducer brings about the rapid synthesis of interferon mRNA, which is largely complete by 3 hr after the beginning of induction (5, 13, 16, 17). Indirect evidence suggests that interferon mRNA has a half-life of 12–15 hr at 37° in the presence of inhibitors of RNA synthesis (12, 13).

Concurrently with the induction of the synthesis of interferon mRNA, poly(I)·poly(C) is thought also to bring about the synthesis of an RNA species, and possibly also of a protein species, that is responsible for posttranscriptional repression of interferon mRNA. This repressor mechanism is thought to be responsible for the rapid shutoff of interferon production by 6–8 hr after induction despite the intrinsic stability of interferon mRNA (7, 16, 18). It has been proposed that inhibitors of macromolecular synthesis, given at appropriate times, interfere with the synthesis of components of the shutoff mechanism and hence are able to enhance interferon yield (5, 7, 9, 10, 12, 14, 15, 18, 19). The molecules mediating the shutoff appear to be unstable and to have an overall lifetime of only 3–4 hr (7, 16). Furthermore, the shutoff mechanism appears to inactivate interferon mRNA in an irreversible manner (7). It has been suggested that a translational repressor of the kind hypothesized by McAuslan (20) and by Tomkins (21–23) is responsible for the shutoff of poly(I)·poly(C)-induced interferon production in human diploid fibroblasts (7, 9, 14, 15).

It has been discovered recently that polyadenylated RNA extracted from interferon-producing human or mouse cells can be translated in wheat-germ, mouse Ehrlich ascites, Krebs-II ascites, and rabbit reticulocyte cell-free translation systems and in the *Xenopus laevis* oocyte, with the production of biologically active and specific interferon (24–26).

We have used the *X. laevis* oocyte injection assay for interferon mRNA (26, 27) to carry out a direct biochemical test of the hypothesis that the shutoff of poly(I)·poly(C)-induced interferon production in FS-4 cells involves the irreversible inactivation of interferon mRNA. The results described in this communication provide strong support for this hypothesis and show that such inactivation can be prevented by 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB), an inhibitor of RNA synthesis (10, 12, 28, 29).

MATERIALS AND METHODS

Cell Cultures. A human diploid fibroblast strain (FS-4) (5, 30) has been used in all experiments (7, 19, 31). Interferon titrations were carried out with a human cell strain trisomic for chromosome 21 (GM258) (32) obtained from the Human Genetic Mutant Cell Repository, Camden, NJ.

Abbreviations: poly(I)·poly(C), poly(inosinic acid)·poly(cytidylic acid); DRB, 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole; P_i/NaCl, phosphate-buffered saline.

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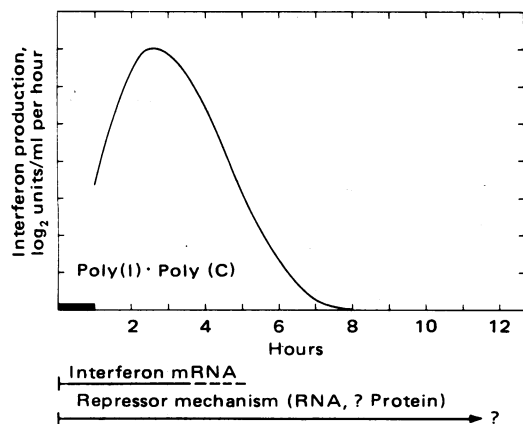


FIG. 1. Scheme of transcription of interferon mRNA and repressor RNA-determinants of interferon synthesis in a culture of FS-4 cells induced with poly(I)·poly(C).

Interferon Induction and Titration. Interferon was induced by exposing confluent FS-4 cultures, washed once with warm phosphate-buffered saline [P_i /NaCl (ref. 33)], to a solution of poly(I)·poly(C) (12.5 or 20 μ g/ml, P-L Biochemicals, Inc.) in Eagle's minimum essential medium for 1 hr at 37°. Interferon was assayed by means of the semimicro method using GM258 cells (30, 34). Interferon titers are expressed in terms of the 69/19 reference standard. One reference unit/ml titrated at 2 units/ml in the assay we used.

Cell Fractionation and RNA Extraction. Cultures were rinsed four times with ice-cold P_i /NaCl, and the cells were scraped into cold P_i /NaCl and pelleted in a refrigerated centrifuge (1000 rpm, 3 min). The cell pellet was either extracted with phenol directly or the nuclear and cytoplasmic fractions were isolated first and then extracted with phenol. For fractionation, the cells were resuspended in one-third-strength reticulocyte standard buffer (3.3 mM NaCl/0.5 mM $MgCl_2$ /3.3 mM Tris-HCl, pH 7.4) for 10 min and the cells were broken in a Dounce homogenizer. Nuclear and cytoplasmic fractions were separated by centrifugation (2000 rpm, 3 min) and the nuclear pellet was further washed with detergent (0.5% deoxycholate/1.0% Tween 40) before RNA extraction (35, 36). RNA from the cytoplasmic fraction was phenol extracted at neutral pH and room temperature whereas that from the nuclear fraction and from whole cells was extracted at low pH (pH 5.1 in acetate buffer) at 65° by procedures described earlier (29, 37–39). Polyadenylated RNA was isolated by poly(U)-Sephadex chromatography (37–39).

Translation of mRNA in Oocytes of *X. laevis*. Oocytes were obtained by dissection of mature *X. laevis* females purchased from Nasco (Fort Atkinson, WI). Oocytes were used within 2 days of dissection. Clusters of approximately five fully developed oocytes were microinjected with 100–150 nl per oocyte of the appropriate RNA dissolved in distilled water or in injection buffer (88 mM NaCl/15 mM Tris-HCl, pH 7.6; ref. 40). Ten to 15 oocytes were usually injected per variable. Five injected oocytes were incubated per 0.1 ml of sterile modified Barth's medium (40) at room temperature (21–23°). At the end of the incubation, the oocytes were broken by means of a capillary pipette in the same aliquot of medium that was used for incubation. The homogenate was clarified by centrifugation for 30 min at top speed in a Beckman Microfuge at 4° and two 50- μ l aliquots of the clarified supernate were used to make up a 2-fold dilution series to test for interferon in duplicate assays.

Cell-Free Translation of mRNA. This was carried out in the wheat germ and HeLa cell systems by using previously described procedures (24, 41, 42).

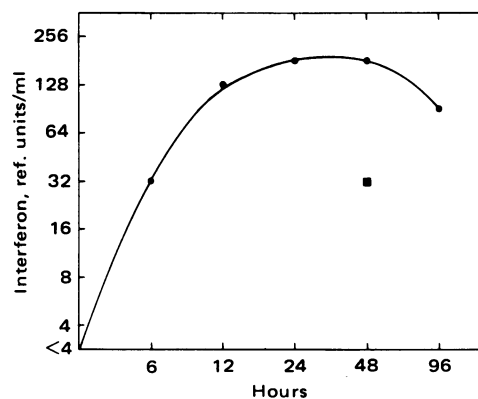


FIG. 2. Time-course of synthesis of interferon in oocytes injected with interferon mRNA. Cytoplasmic polyadenylated RNA (●) (see Table 1) was injected into 50 oocytes (80 ng of RNA per oocyte). An equivalent amount of nuclear polyadenylated RNA (■) (Table 1) was also injected into 15 oocytes (40 ng of RNA per oocyte). Groups of 10 injected oocytes were harvested at different times after injection and the amount of interferon synthesized was determined.

Interferon Neutralization Test. Specific antisera directed against human fibroblast interferon (anti-F antiserum) and human leukocyte interferon (anti-Le antiserum) were a gift from E. A. Havell, New York University School of Medicine, New York, NY. Interferon neutralization by antiserum was tested as described (3).

RESULTS

Cell-Free Translation of Interferon mRNA. Our initial efforts were directed at establishing a reproducible cell-free translation assay for interferon mRNA based on a bioassay (30, 34, 43) to detect interferon synthesis. Polyadenylated total cellular RNA as well as cytoplasmic and nuclear poly(A)-containing RNA were obtained from gram quantities of FS-4 cells that had been treated with poly(I)·poly(C) in the presence of cycloheximide for 4 hr (25, 26). The mRNA preparations were translated, together with control preparations of rabbit globin and dog pancreas mRNAs, in the wheat-germ and HeLa translation systems by using previously described procedures (24–26, 41, 42). It was our uniform experience that mRNA preparations from induced FS-4 cells stimulated incorporation of radioactive amino acids in both systems to an extent comparable to the stimulation produced by rabbit globin and dog pancreas mRNAs (approximately 25-fold in the wheat-germ system and 7-fold in the HeLa system). Nevertheless, no interferon activity was detectable in the translation products (<10 reference units/ml). Varying the concentration of spermine in the translation system and adding a membrane fraction from dog pancreas (44, 45) did not alter this result. Inasmuch as the *same* cytoplasmic and nuclear RNA preparations were efficiently translated into biologically active human fibroblast interferon by *X. laevis* oocytes 6 months later (Figs. 2 and 3; Table 1), it appears that synthesis of the interferon polypeptide is not by itself sufficient to generate biologically active interferon.

Translation of Interferon mRNA in Oocytes of *X. laevis*. The *same* sample of polyadenylated cytoplasmic mRNA obtained from induced FS-4 cells that had been used in some of the cell-free translation experiments was used to obtain data presented in Table 1 and Figs. 2 and 3. Oocytes were injected with mRNA and incubated for 48 hr. The clarified oocyte homogenates were tested for interferon activity. As can be seen in Table 1, *X. laevis* oocytes synthesized biologically active interferon in high yield after injection of mRNA obtained from poly(I)·poly(C)-induced FS-4 cells. Interferon activity was neutralized by antiserum against human fibroblast interferon

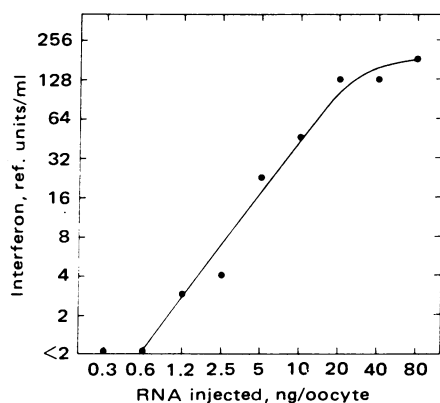


FIG. 3. Relationship between the amount of interferon mRNA injected per oocyte and the interferon yield. A serial 2-fold dilution of the interferon mRNA stock (Table 1) was prepared in injection buffer and 15 oocytes were injected with about 150 nl each of the various RNA dilutions. Interferon synthesized by the end of 48 hr was determined.

but not by that against human leukocyte interferon. The oocyte supernate was inactive in the interferon assay in mouse L cells. In separate experiments, we have observed that control uninjected or mock injected oocytes as well as oocytes injected with mRNA from uninduced FS-4 cells do not yield detectable interferon activity (see Fig. 4).

The time-course of synthesis of interferon in oocytes after injection of cytoplasmic polyadenylated mRNA is described in Fig. 2. It can be seen that interferon was detectable within 6 hr of mRNA injection. Maximal interferon yields were obtained after 24–48 hr of incubation. In additional experiments, maximal yields were obtained around 32 hr after injection (data not shown). Total cellular polyadenylated RNA from induced cells gave similar results (data not shown). An incubation period of 48 hr was therefore chosen for subsequent experiments.

It is noteworthy that nuclear poly(A)-containing RNA, when injected into oocytes, resulted in the synthesis of interferon to a level approximately 15–20% of that obtained with cytoplasmic RNA (Fig. 2). Appropriate control experiments (not shown) indicated that these molecules did not represent cytoplasmic contamination of the nuclear RNA preparation.

The dependence of interferon synthesis in oocytes on the amount of mRNA injected is illustrated in Fig. 3. Detectable interferon yields were obtained by injection of approximately 1 ng of RNA per oocyte. There was a linear increase in the interferon yield with increasing amounts of injected RNA in the range of 1.2–20 ng of RNA. A maximum titer of 192 reference units/ml was obtained by injecting 80 ng of RNA per oocyte. The leveling off of the dose-response curve above 20 ng may be the result of injecting sufficient mRNA to saturate the translational capacity of the oocytes. Alternatively, the leveling off may be due to saturation of the posttranslational modification machinery that may operate in the synthesis of biologically active interferon in oocytes (46). The reproducibility of this assay is demonstrated by the observation that the same mRNA preparation gave identical interferon yields in three separate experiments (80 ng of RNA injected per oocyte, followed by incubation for 48 hr, gave 192 reference units/ml in experiments summarized in Table 1 and Figs. 2 and 3). Our overall experience indicates that variation in the oocyte assay is no greater than variation in the interferon titrations (a 2-fold error). We have used the oocyte assay to measure the concentration of biologically active interferon mRNA during different stages of interferon production in FS-4 cells.

Synthesis and Inactivation of Interferon mRNA in Human Cells. The extraction of polyadenylated mRNA and its translation in *X. laevis* oocytes, described in the preceding

Table 1. Synthesis of human fibroblast interferon in oocytes

Condition of interferon assay	Interferon titer, ref. units/ml
Human GM258 cells	192
Human GM258 cells + anti-F antiserum	<8
Human GM258 cells + anti-Le antiserum	192
Mouse L cells	<4*

Confluent FS-4 cells in 17 roller bottles (490 cm² each; Corning) were exposed to poly(I)-poly(C) (12.5 μg/ml in Eagle's minimum essential medium) in the presence of cycloheximide (50 μg/ml) for 4 hr. At the end of this period, the cultures were washed four times with ice-cold P_i/NaCl and the cells were harvested by scraping. The cell pellet (about 1 ml packed volume) was resuspended in one-third-strength reticulocyte standard buffer and the cytoplasmic and detergent-washed nuclear fractions were isolated. RNA in the two fractions was obtained by phenol extraction, and poly(A)-containing RNA was selected by poly(U)-Sephacrose chromatography. Polyadenylated RNA was dissolved in 100 μl of distilled water. Each of 30 oocytes was injected with approximately 80 ng of cytoplasmic poly(A)-containing RNA (about 150 nl per oocyte) and the oocytes were incubated for 48 hr in modified Barth's medium in a total volume of 0.6 ml. The oocytes were then homogenized in the same medium and aliquots of the clarified homogenate were assayed for interferon. Homogenates from mock-infected oocytes did not contain detectable amounts of interferon (also see Fig. 4). The sensitivity of the L cells to mouse interferon and the specificity of the antisera were verified in separate control assays.

* A dilution of 1:4 was tested.

section, was scaled down for smaller quantities of cells. Ten 150-mm petri-dish cultures provided enough cells (about 0.3 ml packed volume) to allow the isolation of sufficient quantities of total cellular polyadenylated RNA. Answers to two key questions were sought. First, did the shutoff of interferon production involve a loss of translatable interferon mRNA? Second, what effect did superinducing regimens such as the presence of DRB throughout the production period have on interferon mRNA content?

Fig. 4 and Table 2 present a summary of the results obtained. As reported earlier (see the introduction), poly(I)-poly(C)-induced FS-4 cells showed a burst of interferon synthesis that peaked at approximately 3 hr after induction and was shut off by 6–8 hr (Fig. 4). In the presence of 40 μM DRB, the rate of interferon production showed a distinct lag and then continued at a high level for an extended period of time (up to 4 days) (12, 13). We determined the relative content of interferon mRNA in uninduced cultures, in induced cultures near the peak of production (2.5 hr), in induced cultures in the shutoff phase (6 hr), and in cultures induced and maintained in 40 μM DRB for 6 hr. It is clear from Fig. 4 and Table 2 that the shutoff of interferon production was accompanied by a loss or inactivation of translatable interferon mRNA in induced cells. A similar result was obtained when polyadenylated RNA from the cytoplasmic fraction was tested for interferon mRNA activity. The presence of DRB in the cultures prevented this loss or inactivation (Fig. 4 and Table 2). The data in Table 2 show that the low yield of interferon obtained in oocytes injected with mRNA preparations from cultures in the shutoff phase was not an experimental artifact because mRNA samples from uninduced cultures or from cultures in the shutoff phase did not interfere with the translation of known amounts of interferon mRNA.

Table 2 also shows that RNA from interferon-producing cells that did not bind to poly(U)-Sephacrose had no interferon mRNA activity. However, these flow-through RNA preparations did interfere with the translation of added interferon mRNA, presumably because of their high content of ribosomal RNA and contaminating DNA. This result suggests that it may be

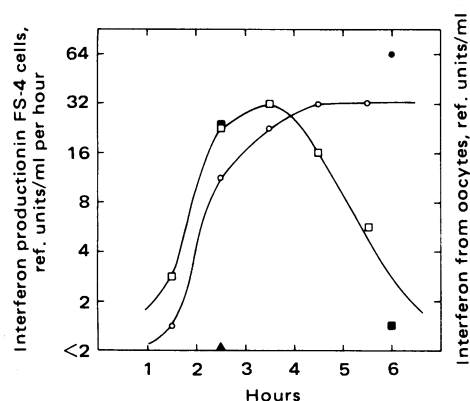


FIG. 4. Correlation between interferon production and interferon mRNA content of FS-4 cells during induction, shutoff, and superinduction. Ten 150-mm Falcon petri-dish cultures of confluent FS-4 cells were used for each analysis. The cultures were induced with poly(I)-poly(C) (20 μ g/ml) in 10 ml Eagle's minimum essential medium for 1 hr starting at "0 hour." One batch of cultures did not receive poly(I)-poly(C) whereas another batch received poly(I)-poly(C) together with 40 μ M DRB. The DRB-treated batch was maintained in DRB throughout the experiment. Two of the induced cultures were used to monitor the rate of interferon production by hourly medium (10 ml) change; one culture was from the DRB-free batch (\square) and the other was treated with DRB (\circ). DRB-containing samples were dialyzed prior to the assay for interferon. At 2.5 hr, a batch of induced (\blacksquare) and a batch of uninduced (\blacktriangle) cultures were harvested, and total cellular RNA was fractionated into polyadenylated RNA and flow-through RNA by using a poly(U)-Sephadex column. Another batch of induced cultures (\blacksquare) and the DRB-treated cultures (\bullet) were similarly harvested at 6 hr after induction. The polyadenylated RNA and the flow-through RNA were ethanol precipitated and dissolved in 20 and 100 μ l, respectively, of injection buffer. Approximately 2 μ l of each polyadenylated RNA preparation was injected into 13–15 oocytes (30 ng of RNA per oocyte) and interferon synthesis was monitored.

premature to conclude that the poly(A)-lacking fraction does not contain any interferon mRNA (24–26).

DISCUSSION

In previous reports, we have systematically evaluated several different hypotheses that might explain superinduction of human interferon production (for reviews see refs. 10 and 11). It has become apparent, on the basis of indirect experiments, that the enhancement of interferon production in FS-4 cells by inhibitors of RNA or protein synthesis is not related to an inhibition of intralysosomal protein degradation (19) or to an inhibition of mRNA competition during the shutoff phase (7). However, evidence has been obtained, by using inhibitors such as DRB, to implicate a posttranscriptional regulatory event in the shutoff of human interferon production, mediated by a rapidly turning over RNA and possibly by protein (9, 11–13, 17, 18, 47). These data are generally consistent with the hypothesis that the shutoff of poly(I)-poly(C)-induced interferon synthesis in FS-4 cells is mediated by a translational repressor of the McAuslan–Tomkins type (14, 15, 20–23). McAuslan (20) and Tomkins and his colleagues (21–23) suggested the possibility that translation of mRNA in eukaryotic cells might be regulated in a specific manner by rapidly turning over RNA and protein molecules that serve to repress, inactivate, or degrade mRNA. In the present study, we have carried out a direct biochemical test of this hypothesis in the interferon system.

We have demonstrated that interferon mRNA is rapidly inactivated or degraded during the shutoff phase but is protected in the presence of DRB, a selective inhibitor of nuclear heterogeneous RNA and mRNA synthesis (28, 29, 48). The fact that interferon mRNA is inactivated during the shutoff phase in FS-4 cells has also been observed by R. L. Cavalieri, E. A.

Table 2. Interferon mRNA activity of RNA preparations

Cell cultures used	Interferon titer, ref. units/ml	
	Poly(A) ⁺ RNA	Flow-through RNA
Uninduced, 2.5 hr	<4	<4
Induced, 2.5 hr	48	<4
Induced, 6 hr	4	<4
Induced, DRB-treated, 6 hr		<4
Alone	128	
+ Uninduced, 2.5 hr, poly(A) ⁺ RNA	48 (64*)	
+ Induced, 6 hr, poly(A) ⁺ RNA	192 (64*)	
Induced, cycloheximide-treated, 4 hr		
Alone	384	
+ Uninduced, 2.5 hr, flow-through	<4 (192)	
+ Induced, 6 hr, flow-through	<4 (192)	

RNA preparations described in this table came from the experiments presented in Fig. 4 and Table 1. Approximately 30 ng of polyadenylated RNA [poly(A)⁺ RNA] or 500 ng of flow-through RNA was injected per oocyte. In carrying out the mixing controls, equal volumes (2 μ l) of the appropriate samples were mixed and then injected into oocytes. Hence, interferon yields from injecting mixtures would be half of those obtained by injecting interferon-mRNA-containing samples alone. Expected titers are indicated in parentheses. Interferon mRNA from induced, cycloheximide-treated cells was cytoplasmic polyadenylated RNA as described in Table 1.

* Deviations indicate experimental variation and are not significant, as demonstrated by other experiments.

Havell, S. Pestka, and J. Vilček (personal communication) using the oocyte assay. These results differ from those reported recently by Raj and Pitha (49). The observation that interferon mRNA is inactivated or degraded during the shutoff phase is entirely consistent with the McAuslan–Tomkins repressor hypothesis.

A proper interpretation of the data presented in this communication requires that the bioassay for interferon correctly measure the quantity of interferon protein synthesized. At the present time, the validity of the bioassay cannot be rigorously proven. Nevertheless, Fig. 3 shows that a linear relationship exists between the amount of mRNA injected into oocytes and the interferon yield obtained over the range of 1–20 ng of RNA injected per oocyte. This relationship (slope about 45°) suggests that the synthesis of biologically active interferon in the oocyte translation assay correctly reflects the amount of interferon synthesized, at least over this range of injected mRNA.

We have confirmed that oocytes produce active interferon that possesses the same species specificity and the same immunological characteristics as interferon synthesized by cells from which the mRNA was extracted (26, 27). Furthermore, it has been shown by other investigators that, when injected into *X. laevis* oocytes, interferon mRNA obtained from FS-4 cells brings about the synthesis of interferon which has an apparent molecular weight of 21,000 (26, 27).

It is known that oocytes can correctly cleave, acetylate, phosphorylate, and hydroxylate polypeptides synthesized in response to injected mRNA (40, 50). In preliminary experiments with rat growth hormone mRNA (P. M. Süssman, P. B. Sehgal, and C. Bancroft, unpublished data), we have observed that *X. laevis* oocytes accumulate the processed growth hormone polypeptide and not pregrowth hormone (51). It appears that *X. laevis* oocytes efficiently accomplish the posttranslational modifications of the interferon molecule necessary to generate biologically active interferon, at least over the linear portion of the curve presented in Fig. 3. Our failure to observe interferon synthesis in cell-free translation systems, such as the

wheat-germ system, may reflect the inability of our cell-free systems to perform these modifications.

The loss of translatable interferon mRNA (Fig. 4 and Table 2) from cells in the shutoff phase indicates that the shutoff of interferon production is not due to simple displacement of interferon mRNA from polysomes by competing cellular mRNA (52). It is apparent that interferon mRNA does not survive in FS-4 cells in an untranslated but phenol-extractable state as has been observed during the shutoff of vitellogenin synthesis in avian liver after a primary stimulation with estrogen (53). The data obtained in the interferon system are also quite different from those reported by Killewich *et al.* (54). These investigators showed that the concentration of the mRNA for superinducible tryptophan 2,3-dioxygenase, measured in a Krebs ascites cell-free translation system during superinduction of the enzyme activity by actinomycin D, was identical to that in actinomycin D-free controls. The interferon mRNA content of cells superinduced with DRB is markedly higher than that of comparable cultures 6 hr after induction. It appears very likely that the regulation of human interferon production involves a McAuslan-Tomkins type translational repressor which inactivates or degrades interferon mRNA.

Note Added in Proof. We have observed that the rate of interferon production and the concentration of translatable interferon mRNA in FS-4 cells induced and maintained in the presence of DRB decay with virtually identical half-lives. Thus, continued interferon production in DRB-treated cells can be entirely explained in terms of the level of functional interferon mRNA.

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