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Induction and Maintenance of 2′,5′-Oligoadenylate Synthetase in Interferon-Treated Chicken Embryo Cells

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Treatment of primary cultures of chicken embryo cells with homologous interferon results in a substantial increase in the level of 2′,5′-oligoadenylate synthetase activity that can be detected in cell extracts. This increase can be prevented by inhibitors of RNA or protein synthesis and is thus thought to represent the induction of an interferon-inducible gene, perhaps the 2′,5′-oligoadenylate synthetase gene itself. To examine this response in greater detail, we studied its kinetics under the following conditions: (i) cessation of interferon treatment after different lengths of time, (ii) delayed inhibition of RNA or protein synthesis, and (iii) combinations of these treatments. The results showed that in cells treated continuously with interferon, the enzyme level reached a peak after 9 h of treatment and then decreased with a half-life of about 30 h, despite the continued presence of interferon. Removal of interferon during induction reduced the peak level of activity that was attained and somewhat accelerated its decline but did not otherwise affect the time-course of the response. On the other hand, removal of interferon after maximum induction clearly accelerated the decay of enzyme activity. This process could be delayed by inhibitors of protein synthesis, which effectively stabilized the induced enzyme. This behavior is reminiscent of other inducible enzymes, such as the steroid-induced tyrosine aminotransferase, and suggests that the level of 2′,5′-oligoadenylate synthetase, which is also inducible by steroid hormones in some cell types, is subject to similar control mechanisms.

Interferons exert most of their effects on homologous cells by inducing the expression of certain cellular genes that would otherwise remain repressed (36). The products of these interferon-inducible genes are thought to be responsible, directly or indirectly, for the many biological consequences of interferon treatment. However, the conclusive identification of even a single interferon-inducible gene is incomplete. The best candidate is the gene that encodes the enzyme 2′,5′-oligoadenylate (2-5A) synthetase (6, 8, 17). This unusual enzyme activity increases from 10- to 5,000-fold upon interferon treatment of a wide variety of cells (6, 35). The increase requires the de novo synthesis of RNA and protein and depends on the interferon dose and the duration of treatment. Moreover, in chicken embryo cells (CEC), a 56,000-dalton polypeptide that has been tentatively identified as the chick enzyme is synthesized specifically in response to interferon treatment (4). Finally, mRNA extracted from interferon-treated mouse L-cells has been translated by injection into *Xenopus* oocytes to produce active 2-5A synthetase, whereas mRNA from untreated L-cells yields no active enzyme (34). These data argue strongly that 2-5A synthetase is the product of an interferon-inducible gene.

When activated by double-stranded RNA (dsRNA), this enzyme catalyzes the reaction: nATP $\rightarrow$ ppp5′A(2′p5′A)$_{n-1}$ + $(n-1)$PP$_i$. The reaction products constitute an oligomeric series of molecules that extends up to the 5-mer in vivo (20) and up to the 15-mer in vitro (13). The trimer and higher oligomers activate a latent endoribonuclease which can then cleave single-stranded RNAs such as mRNA and certain presumably exposed regions of rRNA (3, 12, 39).

The precise role that is played by the 2-5A system in the response of cells to interferon or in the metabolism of untreated cells is not yet clear, but many of its components are subject to complex regulation. For example, the 2-5A synthetase level changes not only upon interferon treatment but also in response to some hormones and to changes in the growth state of some cells (9, 18, 21, 23, 30, 35). For catalytic activity, the enzyme requires activation. In vi-

tro, this can be accomplished by dsRNA, and it has therefore been suggested that dsRNA of viral origin is a physiological activator of the enzyme in infected cells (27). The increase in 2-5A levels that follows encephalomyocarditis virus infection of untreated or interferon-treated cells is consistent with this suggestion (20). However, the presence of the enzyme in the nuclei as well as the cytoplasms of interferon-treated cells and the ability of the enzyme to be activated by heterogeneous nuclear RNA reinforce the possibility that the 2-5A system plays a more complex role in RNA metabolism (28, 29). Furthermore, the product of the activated 2-5A synthetase, 2-5A itself, is rapidly degraded by a 2'-phosphodiesterase, another enzyme activity that is elevated by interferon treatment of some cells (26, 33, 38). Finally, the 2-5A-dependent endonuclease can be inactivated by an unknown mechanism during virus infection of non-interferon-treated cells (10).

Clearly, elucidating the regulation of the 2-5A system presents a major challenge, and as a first step, we have examined in detail the induction of 2-5A synthetase in interferon-treated CEC. The results presented in this paper define the time-course of enzyme induction, show that the maintenance of induced enzyme levels requires continuous exposure to interferon, and demonstrate that the removal of interferon from induced cells leads to an accelerated loss of enzyme activity via a protein synthesis-dependent process. These observations suggest the existence in cells of a mechanism for the inactivation of 2-5A synthetase at the end of a period of interferon treatment.

MATERIALS AND METHODS

Cells. Primary cultures of CEC were prepared from 10-day-old embryos as previously described (7) and cultured at a density of 10^7 cells per 60-mm plate in NCI medium (GIBCO Laboratories supplemented with 6% calf serum. The cultures were maintained at 37°C in a 5% CO_2 atmosphere and used for experiments between 2 and 5 days after plating.

Interferon. Chicken interferon was prepared from primary cultures of CEC cultivated for 7 days in NCI medium with 6% calf serum and then infected with Sindbis virus at a multiplicity of 1 PFU per cell. After virus adsorption (1 h at 37°C), the inoculum was removed and replaced with NCI medium without serum. The cultures were incubated for 24 h at 40.5°C, and then the medium was collected and centrifuged at 1,000 rpm for 10 min to remove cells. The pH of the supernatant was adjusted to 2 with 2 N $HClO_4$, and the preparation was kept overnight at 4°C. After centrifugation at 1,500 rpm for 30 min, the pH of the supernatant was readjusted to 7 with 5 N NaOH, and the supernatant was stored at −70°C. The interferon titer of the supernatant was determined on 4-day-old primary cultures of CEC challenged with vesicular stomatitis virus. In the experiments reported here, the interferon preparations had specific activities of 0.9×10^6 to 3.0×10^6 50% plaque reduction units per mg of protein and were used at a final concentration of 125 U/ml to treat cells.

Interferon removal and inhibitor treatment. For the interferon removal experiments, media containing interferon were aspirated from the plates, which were then washed two times with phosphate-buffered saline at 37°C. Media without interferon were added, and incubation at 37°C in 5% CO_2 was continued. RNA synthesis was inhibited by the addition of actinomycin D (Sigma Chemical Co.) or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB; Calbiochem). Actinomycin D was prepared as a stock solution at 1 mg/ml in 100% ethanol and used at a final concentration of 0.5 μg/ml. At this level, RNA synthesis was inhibited by 94% at 6 and 12 h after addition and by 83% at 24 h after addition; protein synthesis was inhibited by 0, 12, and 36% at 6, 12, and 24 h, respectively. However, the cytopathic effect of the drug became quite severe after 15 h of treatment. DRB was prepared as a 10 mM stock solution in 50% ethanol and used at a final concentration of 75 μM. Under these conditions, RNA synthesis was inhibited 82, 87, and 91% at 6, 12, and 24 h, respectively, after the start of treatment, and very little cytopathic effect was evident. Protein synthesis was inhibited by the addition of cycloheximide (Sigma), which was prepared as a stock solution in phosphate-buffered saline and used at a final concentration of 50 μg/ml. Protein synthesis was inhibited by more than 99% at all times up to 36 h after addition of this concentration. After removal of cycloheximide, the rate of protein synthesis recovered to >60% of the uninhibited value within 2 to 3 h.

Preparation of cell extracts. Cells were lysed on the plates essentially as described by Ball (4) with a slightly modified lysis buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Sigma; pH 7.6], 50 mM KCl, 5 mM magnesium acetate, 1 mM dithiothreitol, 0.5% Nonidet P-40 [BDH]). Two 0.2-ml samples of lysis buffer were added per 60-mm plate; the contents were then collected, pooled, and centrifuged for 2 min in an Eppendorf Microfuge. The supernatants were further fractionated by centrifugation at 100,000 × *g* for 2 h at 4°C. The postribosomal supernatants (S100s) thus obtained were stored at −70°C and used for the assay of 2-5A synthetase activity.

2-5A synthetase assay. S100s were assayed for 2-5A synthetase activity as described by Ball (5). The dsRNA-dependent incorporation of [^{3}H]ATP into 2-5A was measured in 10-μl reaction mixtures containing 50 mM magnesium acetate, 1.5 mM [^{3}H]ATP (Amersham Corp.; 16.7 Ci/mol), 10 μg of polyinosinic acid · polycytidylic acid [poly(I) · poly(C); P-L Biochemicals, Inc.] per ml, and 5 μl of S100. The reaction mixtures were incubated at 30°C for 2 h and then heated at 100°C for 5 min. Denatured protein was removed by centrifugation for 2 min in an Eppendorf Microfuge, and 7.5-μl samples of the supernatants were transferred to fresh reaction tubes, each of which contained 2.5 μl of bacterial alkaline phosphatase (Sigma; type III) to give a final enzyme concentration of 9 to 10 U/ml. After a 2-h incubation at 37°C, 6-μl samples of the reaction mixtures were spotted onto squares of DEAE-cellulose paper (1 cm^2; Whatman DE81). By this procedure, unreacted ATP was digested to adenosine which failed to bind to the DEAE-

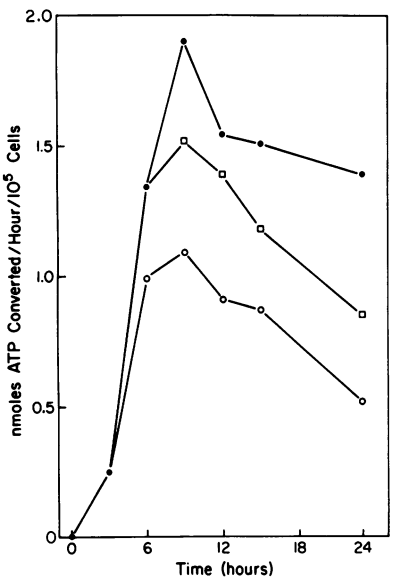

FIG. 1. Time course of 2-5A synthetase induction after removal of interferon. Confluent monolayers of CEC were treated with 125 U of interferon per ml either continuously (●) or for only 3 h (○) or 6 h (□) before the interferon was removed. At the times shown, the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured.

cellulose paper; digestion of 2-5A, on the other hand, yielded phosphatase-resistant cores which were quantitatively retained on the filters. The filters were dried, washed three times by gentle agitation in distilled water, and then placed in scintillation vials containing 0.3 or 0.5 ml of 0.3 M KOH. After elution for about 1 h, the samples were counted in Aquasol (New England Nuclear Corp.) at 4°C. Duplicate reaction mixtures that lacked poly(I) · poly(C) were assayed for each S100, and the incorporated radioactivity was subtracted from the values observed in the presence of poly(I) · poly(C).

Enzyme activities are presented as nanomoles of ATP incorporated into 2-5A per hour per 10^5 cells. As can be seen from the results, minor variations were observed in the 2-5A synthetase levels that could be induced in different primary cultures, but the reproducibility within each culture was ±6%.

RESULTS

Time-course of 2-5A synthetase induction. The time-course of induction of 2-5A synthetase was measured after treatment of CEC with 125 U of interferon per ml (Fig. 1). As observed previously (4), there was a short lag after which the enzyme level increased to a peak at about 9 h. During the next 15 h, the enzyme activity declined with a half-life of about 30 h. In our previous work, using interferon doses of 250 U/ml, we did not observe a decline of 2-5A synthetase activity before 18 h after treatment (4), and at 300 U of interferon per ml, CEC maintained their induced 2-5A synthetase levels for 30 h (25).

To determine whether interferon treatment resulted in cells becoming irreversibly committed to the production of 2-5A synthetase, we examined the effect of removing the interferon during the induction period. No evidence for an irreversible commitment was obtained; interferon treatment for only 3 or 6 h yielded lower peak enzyme activities, but the peaks still occurred 9 h after the start of treatment (Fig. 1). However, the subsequent decline in 2-5A synthetase activity occurred with a half-life of 15 to 18 h, a somewhat more rapid decline than that observed in the continuous presence of interferon. A consequence of the accelerated loss of enzyme activity was that the removal of interferon, even after 9 h of induction, reduced the 2-5A synthetase activity that could be measured in cell extracts at the end of a 24-h induction period (Fig. 2).

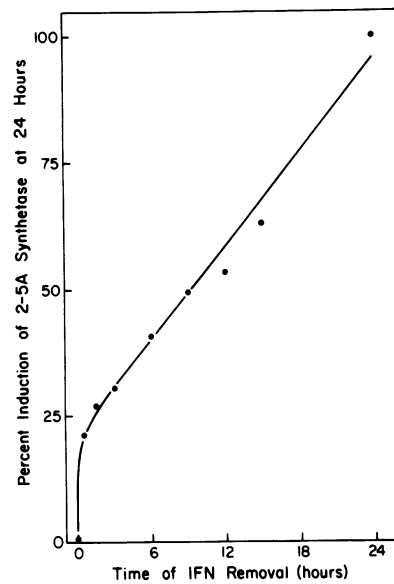

FIG. 2. Effect of interferon (IFN) removal on the induction of 2-5A synthetase, measured 24 h after the start of interferon treatment. Confluent monolayers of CEC were treated with 125 U of interferon per ml, and at various times after the start of treatment the media containing interferon were removed, the cell monolayers were washed thoroughly, and fresh media that lacked interferon were added. Incubation at 37°C was continued until 24 h after the start of treatment, when the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured. The results are expressed as percentages of the activity present after 24 h of continuous exposure to interferon. For the zero-time point, media containing interferon were added to the monolayers and removed immediately; the 2-5A synthetase level at 24 h was not significantly different from that in cells which had never been exposed to interferon.

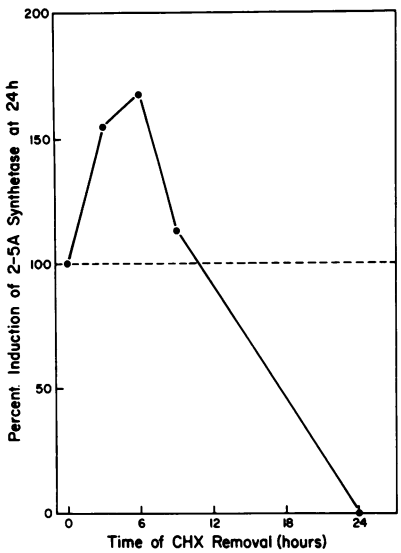

FIG. 3. Effect of inhibition of protein synthesis on the induction of 2-5A synthetase. Confluent monolayers of primary CEC were treated with (per milliliter) 125 U of interferon and 50 μg of cycloheximide (CHX). At the times shown, the cycloheximide inhibition was reversed by removing the media, washing the cells twice with phosphate-buffered saline at 37°C, and adding fresh media that lacked cycloheximide. At 24 h after the start of interferon treatment, the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured. The results are expressed as percentages of the activity present at 24 h of interferon treatment in the absence of cycloheximide.

Inhibition of protein synthesis. Regulation of 2-5A synthetase induction was examined further by adding cycloheximide for different periods during the induction process. As expected, the continuous presence of 50 μg of cycloheximide per ml throughout the period of interferon treatment completely prevented the appearance of 2-5A synthetase activity. However, if the drug was present only during the first 3, 6, or 9 h of treatment, a 50 to 70% overproduction of enzyme activity was observed at 24 h (Fig. 3). Other investigators have described this effect on the induction of 2-5A synthetase and other interferon-inducible gene products in various mammalian cell systems, but in all cases, the extent of overproduction has been no more than twofold (15, 19). Attempts to increase the degree of overproduction by combining an early inhibition of translation with a later inhibition of transcription were unsuccessful (data not shown).

Our initial hypothesis to explain the accelerated decline of 2-5A synthetase activity that occurred upon removal of interferon (Fig. 1) was that the shutoff of enzyme synthesis revealed the true rate of enzyme inactivation or degradation. To test this hypothesis, we examined the effect of inhibiting protein synthesis with cycloheximide after the peak of enzyme activity had been reached. The results failed to confirm the hypothesis because, in the continued presence of interferon, cycloheximide had no effect on the rate of 2-5A synthetase decay (Fig. 4). This indicated either that the enzyme level was not maintained as a dynamic steady state under these conditions or that cycloheximide inhibited both synthesis and decay. The latter possibility was supported by the observation that inhibiting protein synthesis at the time of interferon removal prevented the accelerated decline of activity (Fig. 4). Even 12 h after interferon removal, the addition of cycloheximide significantly reduced the rate of 2-5A synthetase disappearance. Thus, the rapid elimination of 2-5A synthetase that followed interferon removal from CEC required ongoing protein synthesis.

We explored the possibility that the 2′-phosphodiesterase, which is partially interferon inducible in some cell types (19, 33), was responsible for the apparent decline of 2-5A synthetase activity. However, no evidence of interference in the 2-5A synthetase assay was forthcoming from experiments in which cell extracts with peak and postpeak activities were mixed and assayed or from experiments in which 2-5A synthetase levels were measured after partial purification of the enzyme by immobilization on poly(I) · poly(C)-agarose.

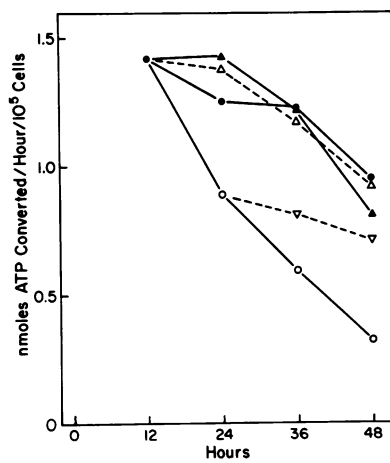

FIG. 4. Effect of inhibition of protein synthesis on the decline of 2-5A synthetase activity in the presence and absence of interferon. Confluent monolayers of CEC were treated with 125 U of interferon per ml either continuously (●, ▲) or for only 12 h (○, △, ▽). Cycloheximide was added at 12 h (▲, △) or at 24 h (▽) after the start of interferon treatment, to a final concentration of 50 μg/ml. At the times shown, the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured.

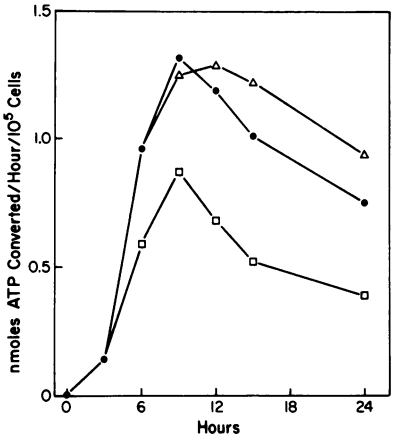

FIG. 5. Time-course of 2-5A synthetase induction after the addition of actinomycin D. Confluent monolayers of CEC were treated with 125 U of interferon per ml (●, □, △), and actinomycin D was added to some plates at either 3 h (□) or 6 h (△) to a final concentration of 0.5 μg/ml. At the times shown, the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured.

Inhibition of RNA synthesis. Continuous exposure of cells to interferon appeared to be necessary to maintain maximum induction of 2-5A synthetase, but it was unclear whether continuous enzyme synthesis was occurring. Consequently, we next examined whether continuous mRNA synthesis was required. It has been established that transcription is necessary for both the development of the antiviral state and the induction of 2-5A synthetase in interferon-treated CEC (4, 24). This requirement was studied more closely by adding actinomycin D to inhibit DNA-dependent RNA synthesis at various times after interferon treatment. When added at 3 h, actinomycin D reduced the level of 2-5A synthetase present at all later times but did not otherwise alter the time-course (Fig. 5). However, the addition of the inhibitor at 6 h or later slightly delayed the peak of activity but had no significant effect on the maximum enzyme level that was attained (Fig. 5 and 6A). Similar results were obtained with DRB, another inhibitor of transcription, although in this case the addition of the drug 6 h after interferon treatment still inhibited enzyme induction by about 20% (Fig. 6B). Thus, inhibition of transcription during the first 6 to 8 h of interferon treatment inhibited enzyme induction, but at later times it had little effect.

To determine whether the induction or the decline of 2-5A synthetase activity was the dominant process, we removed interferon from cells after 3 h of treatment and then added it back at 9 h after the initial exposure. The levels of enzyme activity were compared with those in cells that

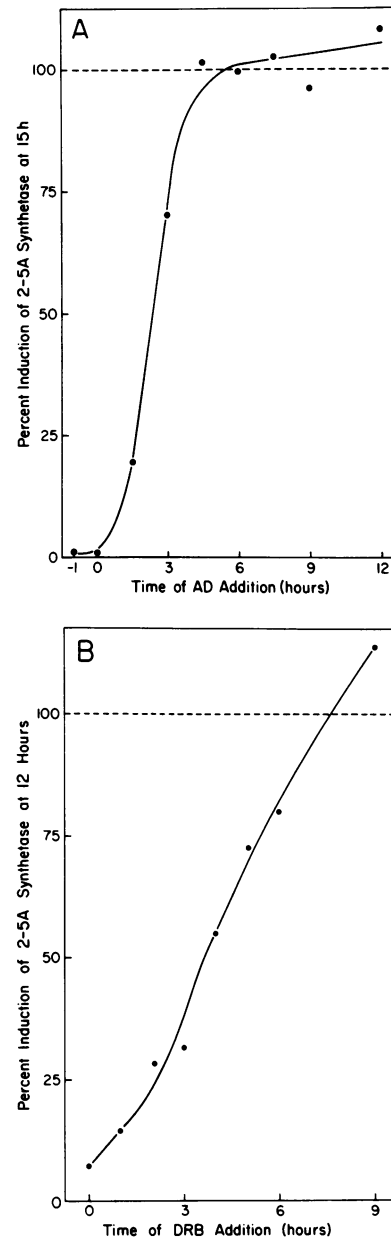

FIG. 6. Effect of inhibition of RNA synthesis on the induction of 2-5A synthetase. Confluent monolayers of CEC were treated with 125 U of interferon per ml. (A) Actinomycin D (AD) was added to the culture medium at the times indicated to a final concentration of 0.5 μg/ml. Incubation at 37°C was continued until 15 h after the start of interferon treatment, and then the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured. The results are expressed as percentages of the enzyme activity present at 15 h in the absence of actinomycin D. (B) DRB was added to the culture medium at the times indicated to a final concentration of 75 μM. Incubation at 37°C was continued until 12 h after the start of interferon treatment, and then the cells were lysed, S100's were

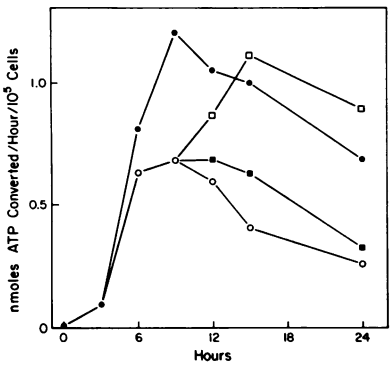

FIG. 7. Effect of interferon removal and readdition on the induction of 2-5A synthetase. Confluent monolayers of CEC were treated with 125 U of interferon per ml either continuously (●) or for only 3 h before the interferon was removed (○, □, ■). Interferon (125 U/ml) was readded at 9 h after the initial exposure (□, ■), and actinomycin D was added simultaneously to some plates to a final concentration of 0.5 μg/ml (■). At the times shown, the cells were lysed, S100s were prepared, and 2-5A synthetase activities were measured. In cells that received actinomycin D at 9 h, the presence or absence of additional interferon did not affect the level of enzyme activity between 9 and 24 h.

were maintained continuously in the presence of interferon and with those in cells that received only the initial 3-h exposure. Enzyme synthesis resumed upon readdition of interferon and peaked at essentially the same level as that reached in cells that had received continuous treatment (Fig. 7). Thus, there was no evidence for a refractory state with respect to 2-5A synthetase induction; enzyme synthesis was dominant over the decline of activity. As expected, enzyme induction upon the readdition of interferon was prevented by the simultaneous addition of actinomycin D.

DISCUSSION

The experiments presented above address the regulation of 2-5A synthetase levels induced by interferon. A thorough analysis of this problem will require quantitative assays for both the 2-5A synthetase mRNA and the polypeptide component(s) of the enzyme. However, although murine 2-5A synthetase mRNA has been successfully translated by injection into *Xenopus* oocytes (34), we have not yet achieved this for the chick enzyme and we therefore lack a quantitative mRNA assay. On the other hand, the catalytic component of the chick 2-5A synthetase has been tentatively identified as a 56,000-molecular-weight polypeptide, and [^{35}S]methionine pulse-labeling studies have shown that this polypeptide is synthesized predominantly during the period from 2 to 10 h after interferon treatment (4). Beyond 10 to 12 h, synthesis of the polypeptide is severely inhibited. Similar transient synthesis of interferon-induced polypeptides has been observed in human fibroblasts (15, 32).

However, isotopic labeling methods are not well suited to an examination of the maintenance and turnover of induced enzymes, so in the work reported here, we relied exclusively on measurements of 2-5A synthetase activity. The inhibitor studies confirmed that both RNA and protein syntheses were necessary for the increase in enzyme level and showed that the required transcription was completed within about the first 6 to 8 h of interferon treatment. The mRNA synthesized during this period appeared to have a functional life of only about 6 h. These values agreed closely with the estimates of the duration of enzyme synthesis that were derived from the earlier pulse-labeling studies (4) and with estimates of the duration of the commitment of L-929 cells to respond to interferon (14). Clearly, enzyme accumulation is a transient process that is limited by unknown mechanisms operating at the level of transcription, translation, or both.

Maintenance of the induced 2-5A synthetase level is a separate issue. If cells were exposed continuously to interferon, their enzyme activity reached a peak after about 9 h and then declined with a half-life of about 30 h. In this respect, the stability of the 2-5A synthetase in CEC was intermediate between the stability of the enzyme in HeLa cells and the stability in quiescent human fibroblasts. In the former case, the enzyme activity appears to be completely stable and is diluted only by cell division after interferon removal (2), whereas in the latter case, the enzyme activity peaks and then decays rapidly despite the continued presence of interferon (22).

The effects of cycloheximide on the maintenance of 2-5A synthetase activity were unexpected. In the continued presence of interferon, cycloheximide did not affect the rate of loss of enzyme activity. After interferon removal, however, the inhibitor effectively stabilized the enzyme by delaying its decline. It seems most likely that under both sets of conditions, the 2-5A synthetase level was regulated by the counteracting elements of synthesis and degradation and that both processes were inhibited by cycloheximide. Although the mechanism remains a mystery, other examples of inhibition by cycloheximide of the degradation of an induced enzyme have been reported (1, 16, 37). The observations that the antiviral activity of interferon can be potentiated by actinomycin D or DRB

prepared, and 2-5A synthetase activities were measured. The results are expressed as percentages of the enzyme activity present at 12 h in the absence of DRB.

(11, 15) and that the virus resistance of interferon-treated CEC decays more slowly after enucleation (31) may be relevant in this context.

Lab et al. (25) have also examined the maintenance of 2-5A synthetase levels in CEC and have reported that the enzyme activity that was lost after interferon removal could be recovered in the presence of cycloheximide. These results contrast with ours, which show only an inhibition of the decay process. We believe that this discrepancy may best be explained by the fact that Lab et al. (25) used a cycloheximide concentration of 20 μg/ml, whereas we used a concentration of 50 μg/ml. Indeed, when we used the lower inhibitor concentration, we also observed recovery of 2-5A synthetase levels (data not shown). On the basis of their results, Lab et al. (25) proposed that 2-5A synthetase is reversibly inactivated by a labile inhibitor made in the absence of interferon. Thus, when protein synthesis is blocked, the inhibitor levels fall and the enzyme recovers its activity. However, our results do not support this hypothesis. The more severe inhibition of protein synthesis imposed by the higher cycloheximide concentration would be expected to allow faster recovery of 2-5A synthetase activity. Instead, the recovery phenomenon was abolished. Furthermore, mixing experiments yielded no evidence for the existence of a reversible inhibitor of 2-5A synthetase (data not shown). Thus, we attribute the recovery of enzyme activity that can be observed at the lower cycloheximide concentration to a partial recovery of 2-5A synthetase synthesis. This interpretation is supported by the observation that 2-5A synthetase synthesis is unusually resistant to translational inhibition (unpublished data).

In summary, these results reveal another level of complexity in the regulation of the 2-5A system. Not only is 2-5A synthetase an inducible, activatable enzyme, but there also appears to be a mechanism for its inactivation or degradation at the end of a period of interferon treatment. This mechanism is at least partially blocked in the presence of interferon, but it is unclear at present if the inactivation process is specific for 2-5A synthetase, directed against all interferon-inducible gene products, or simply a general increase in cellular degradative activity.

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LITERATURE CITED

1. **Auricchio, F., D. Martin, Jr., and G. Tomkins.** 1969. Control of degradation and synthesis of induced tyrosine aminotransferase studied in hepatoma cells in culture. Nature (London) **224**:806–808.
2. **Baglioni, C., P. A. Maroney, and D. K. West.** 1979. 2′5′-Oligo(A) polymerase activity and inhibition of viral RNA synthesis in interferon-treated HeLa cells. Biochemistry **18**:1765–1770.
3. **Baglioni, C., M. A. Minks, and P. A. Maroney.** 1978. Interferon action may be mediated by activation of a nuclease by pppA2′p5′A2′A. Nature (London) **273**:684–687.
4. **Ball, L. A.** 1979. Induction of 2′5′-oligoadenylate synthetase activity and a new protein by chick interferon. Virology **94**:282–296.
5. **Ball, L. A.** 1980. Induction, purification and properties of 2′,5′-oligoadenylate synthetase. Ann. N.Y. Acad. Sci. **350**:486–496.
6. **Ball, L. A.** 1982. 2′, 5′-Oligoadenylate synthetase, p. 281–313. *In* P. D. Boyer (ed.), The enzymes, vol. XV. Academic Press, Inc., New York.
7. **Ball, L. A., and C. N. White.** 1978. Coupled transcription and translation in mammalian and avian cell-free systems. Virology **84**:479–495.
8. **Ball, L. A., and C. N. White.** 1978. Oligonucleotide inhibitor of protein synthesis made in extracts of interferon-treated chick embryo cells: comparison with the mouse low molecular weight inhibitor. Proc. Natl. Acad. Sci. U.S.A. **75**:1167–1171.
9. **Besancon, F., M. F. Bourgeade, J. Justesen, D. Ferbus, and M. N. Thang.** 1981. Two inducers of cell differentiation enhance the 2′5′ oligoadenylate synthetase activity in MSV transformed cells. Biochem. Biophys. Res. Commun. **103**:16–24.
10. **Cayley, P. J., M. Knight, and I. M. Kerr.** 1982. Virus-mediated inhibition of the ppp(A2′p)$_n$A system and its prevention by interferon. Biochem. Biophys. Res. Commun. **104**:376–382.
11. **Chany, C., F. Fournier, and S. Rousset.** 1971. Potentiation of the antiviral activity of interferon by actinomycin D. Nature (London) New Biol. **230**:113–114.
12. **Clemens, M. J., and B. R. G. Williams.** 1978. Inhibition of cell-free protein synthesis by pppA2′p5′A2′p5′A: a novel oligonucleotide synthesized by interferon-treated L cell extracts. Cell **13**:565–572.
13. **Dougherty, J. P., H. Samanta, P. J. Farrell, and P. Lengyel.** 1980. Interferon, double-stranded RNA, and RNA degradation. Isolation of homogeneous pppA(2′p5′A)n-1 synthetase from Ehrlich ascites tumor cells. J. Biol. Chem. **255**:3813–3816.
14. **Gordon, I., and D. Stevenson.** 1980. Kinetics of decay in the expression of interferon-dependent mRNAs responsible for resistance to virus. Proc. Natl. Acad. Sci. U.S.A. **77**:452–456.
15. **Gupta, S. L., B. Y. Rubin, and S. L. Holmes.** 1980. Regulation of interferon action in human fibroblasts: transient induction of specific proteins and amplification of the antiviral response by actinomycin D. Virology **111**:331–340.
16. **Hershko, A., and G. M. Tomkins.** 1971. Studies on the degradation of tyrosine aminotransferase in hepatoma cells in culture. J. Biol. Chem. **246**:710–714.
17. **Kerr, I. M., and R. E. Brown.** 1978. pppA2′p5′A2′p5′A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. Proc. Natl. Acad. Sci. U.S.A. **75**:256–260.
18. **Kimchi, A.** 1981. Increased levels of interferon-induced (2′-5′)oligoisoadenylate synthetase in mature T-lymphocytes and in differentiated Friend-erythroleukemia cells. J. Interferon Res. **1**:559–564.
19. **Kimchi, A., L. Shulman, A. Schmidt, Y. Chernajovsky, A. Fraden, and M. Revel.** 1979. Kinetics of the induction of three translation-regulatory enzymes by interferon. Proc. Natl. Acad. Sci. U.S.A. **76**:3208–3212.
20. **Knight, M., P. J. Cayley, R. H. Silverman, D. H. Wreschner, C. S. Gilbert, R. E. Brown, and I. M. Kerr.**

1980. Radioimmune, radiobinding and HPLC analysis of 2-5A and related oligonucleotides from intact cells. Nature (London) **288**:189–192.

21. **Krishnan, I., and C. Baglioni.** 1980. Increased levels of (2'-5')oligo(A) polymerase activity in human lymphoblastoid cells treated with glucocorticoids. Proc. Natl. Acad. Sci. U.S.A. **77**:6506–6510.
22. **Krishnan, I., and C. Baglioni.** 1981. Regulation of 2',5'-oligo(A) polymerase activity in quiescent human fibroblasts treated with interferon. Virology **111**:666–670.
23. **Krishnan, I., and C. Baglioni.** 1981. Elevated levels of (2'-5')oligoadenylic acid polymerase activity in growth-arrested human lymphoblastoid Namalva cells. Mol. Cell. Biol. **1**:932–938.
24. **Lab, M., and F. Koehren.** 1976. Maintenance and recovery of the interferon-induced antiviral state. Proc. Soc. Exp. Biol. Med. **153**:112–115.
25. **Lab, M., M. N. Thang, K. Soteriadou, F. Koehren, and J. Justesen.** 1982. Regulation of 2-5A synthetase activity and antiviral state in interferon treated chick cells. Biochem. Biophys. Res. Commun. **105**:412–418.
26. **Minks, M. A., S. Benvin, P. A. Maroney, and C. Baglioni.** 1979. Metabolic stability of 2'5'oligo(A) and activity of 2'5'oligo(A)-dependent endonuclease in extracts of interferon-treated and control HeLa cells. Nucleic Acids Res. **6**:767–780.
27. **Nilsen, T. W., and C. Baglioni.** 1979. A mechanism for discrimination between viral and host mRNA in interferon-treated cells. Proc. Natl. Acad. Sci. U.S.A. **76**:2600–2604.
28. **Nilsen, T. W., P. A. Maroney, H. D. Robertson, and C. Baglioni.** 1982. Heterogeneous nuclear RNA promotes synthesis of (2',5')oligoadenylate and is cleaved by the (2',5')oligoadenylate-activated endonuclease. Mol. Cell. Biol. **2**:154–160.
29. **Nilsen, T. W., D. L. Wood, and C. Baglioni.** 1982. Presence of 2',5'-oligo(A) and of enzymes that synthesize, bind and degrade 2',5'-oligo(A) in HeLa cell nuclei. J. Biol. Chem. **257**:1602–1605.
30. **Oikarinen, J.** 1982. Cortisol induces (2'-5') oligoadenylate synthetase in cultured chick embryo tendon fibroblasts. Biochem. Biophys. Res. Commun. **105**:876–881.
31. **Radke, K. L., C. Colby, J. R. Kates, H. M. Krider, and D. M. Prescott.** 1974. Establishment and maintenance of the interferon-induced antiviral state: studies in enucleated cells. J. Virol. **13**:623–630.
32. **Rubin, B. Y., and S. L. Gupta.** 1980. Interferon-induced proteins in human fibroblasts and development of the antiviral state. J. Virol. **34**:446–454.
33. **Schmidt, A., Y. Chernajovsky, L. Shulman, P. Federman, H. Berissi, and M. Revel.** 1979. An interferon-induced phosphodiesterase degrading (2'-5')oligoisoadenylate and the C-C-A terminus of tRNA. Proc. Natl. Acad. Sci. U.S.A. **76**:4788–4792.
34. **Schulman, L., and M. Revel.** 1980. Interferon-dependent induction of mRNA activity for (2'-5')oligo-isoadenylate synthetase. Nature (London) **288**:98–100.
35. **Stark, G. R., W. J. Dower, R. T. Schimke, R. E. Brown, and I. M. Kerr.** 1979. pppA2'p5'A2'p5'A synthetase: convenient assay, species and tissue distribution and variation upon withdrawal of estrogen from chick oviducts. Nature (London) **278**:471–473.
36. **Stewart, W. E., II.** 1981. The interferon system, 2nd ed. Springer-Verlag, New York.
37. **Tomkins, G. M., B. B. Levinson, J. D. Baxter, and L. Dethlefsen.** 1972. Further evidence for post-transcriptional control of inducible tyrosine aminotransferase synthesis in cultured hepatoma cells. Nature (London) New Biol. **239**:9–14.
38. **Williams, B. R. G., I. M. Kerr, C. S. Gilbert, C. N. White, and L. A. Ball.** 1978. Synthesis and breakdown of pppA2'p5'A2'p5'A and transient inhibition of protein synthesis in extracts from interferon-treated and control cells. Eur. J. Biochem. **92**:455–462.
39. **Wreschner, D. H., T. C. James, R. H. Silverman, and I. M. Kerr.** 1981. Ribosomal RNA cleavage, nuclease activation and 2-5A $(ppp(A2'p)_nA)$ in interferon-treated cells. Nucleic Acids Res. **9**:1571–1581.