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Maintenance of Protein Synthesis in Spite of mRNA Breakdown in Interferon-Treated HeLa Cells Infected with Reovirus

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Interferon induces the synthesis of an enzyme which synthesizes 2',5'-oligoadenylate [2',5'-oligo(A)] when activated by double-stranded RNA. The 2',5'-oligo(A) in turn activates an endonuclease (RNase L). Concentrations of 2',5'-oligo(A) sufficient to activate RNase L are formed in interferon-treated HeLa cells infected with reovirus, and a large fraction of cellular mRNA is degraded (T. W. Nilsen, P. A. Maroney, and C. Baglioni, J. Virol. 42:1039–1045, 1982). We report here that in spite of this mRNA degradation, protein synthesis was not significantly inhibited in these cells. When mRNA synthesis was inhibited with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, protein synthesis was markedly decreased, as shown by reduced incorporation of labeled amino acids and a decrease in polyribosomes. This suggested that the turnover of mRNA could be compensated for by increased production of mRNA. The relative concentration of specific mRNAs was measured with cloned cDNA probes. The amount of these mRNAs present in control cells was comparable to that in interferon-treated cells infected with reovirus, whereas it was decreased in the latter cells treated with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole.

Interferon induces the synthesis of an enzyme which polymerizes ATP into oligonucleotides characterized by 2',5'-phosphodiester bonds, collectively designated 2',5'-oligoadenylate [2',5'-oligo(A)] (1). This enzyme, designated 2',5'-oligo(A) polymerase or synthetase, is active only when bound to double-stranded RNA (1). In cell extracts the 2', 5'-oligo(A) polymerase is activated by the addition of synthetic or natural double-stranded RNA, whereas in intact cells it may be activated by the viral double-stranded RNA present in infected cells (1). The 2',5'-oligo(A) produced activates an endoribonuclease (RNase L), which cleaves mRNA (2), viral RNA (16, 17), and rRNA (22). In interferon-treated cells, high levels of 2',5'-oligo(A) polymerase can be activated by double-stranded RNA, and synthesis of 2',5'-oligo(A) has been reported in interferon-treated L cells infected with encephalomyocarditis virus (10, 21) and in HeLa cells infected with reovirus (15).

An endonuclease, presumably RNase L activated by 2',5'-oligo(A), cleaves cellular RNA in interferon-treated HeLa cells infected with reovirus (15). These cells remain viable and are protected from the reovirus infection, since no full-size viral mRNA could be detected by Northern blot analysis (15). The RNA cleavage in these cells was clearly documented by electrophoretic analysis of rRNA and by sedimentation of labeled mRNA on sucrose gradients (15). It was of interest, therefore, to investigate the mechanism(s) employed by these cells to cope with an increased turnover of mRNA. In the present investigation we defined the time after reovirus infection at which mRNA degradation was maximal and examined its effect on cellular protein synthesis. Surprisingly, there was no significant decline in the level of protein synthesis in interferon-treated cells after their infection with reovirus. This finding led us to investigate how these cells maintain a relatively constant level of protein synthesis in spite of extensive mRNA breakdown.

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MATERIALS AND METHODS

Cells and treatment. HeLa cells, obtained from Sheldon Penman, Massachusetts Institute of Technology, were grown in suspension cultures and treated for 16 h with 200 U of human fibroblast interferon per ml (5×10^5 U/mg of protein; obtained from the Interferon Working Group of the National Cancer Institute). Reovirus (Dearing type 3) was amplified on L929 cells and purified as described by Banerjee and Shatkin (3). For infection, HeLa cells were concentrated to 10^7 cells per ml in medium without serum, infected for 60

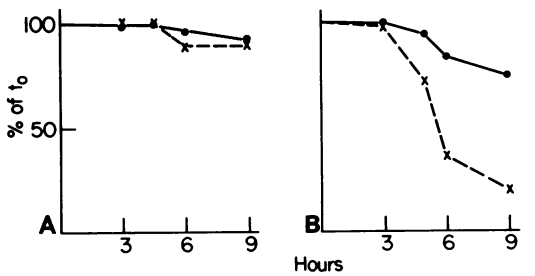

FIG. 1. Decay of cellular mRNA as a function of time after infection with reovirus. Before infection, cellular mRNA was labeled in control (A) or interferon-treated (B) cells during a 3-h incubation with [^{3}H]uridine, as described in the text. The cells were either mock infected (●) or infected with reovirus at an MOI of 20 (X). At the indicated times after infection, culture samples were removed to measure labeled polyadenylate-containing RNA. The amount of this RNA remaining is expressed as a percentage of that obtained from an identical cell sample before infection (t_0).

min at 37°C with reovirus at the indicated multiplicity of infection (MOI), and diluted to 2×10^6 cells per ml in medium containing 5% fetal calf serum.

Labeling and analysis of cellular mRNA. Cell cultures were treated for 30 min with 40 ng of actinomycin D per ml and then incubated for 3 h with 2 μCi of [^{3}H]uridine per ml; the polyadenylate-containing RNA was isolated by chromatography on oligodeoxythymidylate-cellulose (2).

Polysome and protein synthesis analysis. Cell extracts were prepared and sedimented on 15 to 40% sucrose density gradients for 90 min at 40,000 rpm as previously described (15). The absorbance at 260 nm was monitored in a continuous recording spectrophotometer. Protein synthesis was monitored by incubating portions of the cell cultures with [^{3}H]lysine or [^{35}S]methionine as indicated below. The cells were collected by centrifugation and solubilized in 0.5 N NaOH or in 1% sodium dodecyl sulfate. After 10% trichloroacetic acid was added, the precipitate was collected on glass-fiber filters for counting.

Northern blot analysis. Total cellular RNA was prepared by the method of Glisin et al. (7). Cultures of 4×10^7 cells were centrifuged, and the pelleted cells were lysed by the addition of 10 ml of 7 M urea–2% Sarkosyl–1 mM EDTA–50 mM Tris-hydrochloride (pH 7.9). The lysate was homogenized in a Dounce homogenizer to reduce its viscosity, and 4 g of CsCl was added. The sample was then layered over 4 ml of 1.7-g/ml CsCl in 20 mM EDTA and centrifuged at 28,000 rpm for 20 h in the SW41 rotor. The pellet containing the RNA was dissolved in water, precipitated twice with ethanol, and redissolved in water. The RNA was denatured for 15 min at 65°C in 50% formamide–6% formaldehyde–10% glycerol–20 mM MOPS buffer (morpholinopropanesulfonic acid-KOH) (pH 7.0). The denatured RNA was fractionated by electrophoresis for 17 h at 25 V on 1.5% agarose gels containing 6% formaldehyde in 1 mM EDTA, 5 mM sodium acetate, and 20 mM MOPS buffer (pH 7.0). The RNA was transferred to a nitrocellulose sheet as described by Thomas (19), prehybridized for 6 h, and then hybridized with 3×10^6 cpm of nick-translated DNA from plasmid pT1 or pT2 containing cloned α- or β-tubulin cDNA (4) and a plasmid containing cloned cDNA "B" (8). These plasmids were a gift from N. J. Cowan of Princeton University and J. E. Darnell of The Rockefeller University. The plasmids were grown and purified as described by Montgomery et al. (14). The nick-translation followed the procedure described by Maniatis et al. (12).

RESULTS

The mRNA from interferon-treated and untreated HeLa cells was labeled in an incubation with [^{3}H]uridine before the infection with reovirus. mRNA degradation was monitored after infection by chromatography on oligodeoxythymidylate-cellulose (Fig. 1). Little mRNA turnover was observed in uninfected cells or in untreated infected cells. In interferon-treated infected cells, however, about 70% of the labeled mRNA was degraded. This mRNA degradation, therefore, occurred only in cells in which formation of 2′,5′-oligo(A) had been previously detected, and the time of maximum mRNA loss coincided with the peak concentration of 2′,5′-oligo(A) in these cells (15). Since 2′,5′-oligo(A) activates RNase L, it seemed likely that the mRNA was cleaved by this enzymatic activity.

Extensive mRNA degradation was observed in interferon-treated cells between 3 and 6 h after infection with reovirus; in subsequent experiments we investigated the effect of mRNA degradation on protein synthesis at this time postinfection. The protein synthesis activity of these cells was measured by labeling them with [^{3}H]lysine (Table 1). Unexpectedly, incorporation of this amino acid was not significantly inhibited despite the enhanced turnover of mRNA. To rule out the possibility that a change in the lysine pool could account for this pattern of incorporation, we examined protein synthesis by monitoring the polysome profile (Fig. 2). The 80S ribosomes not associated with mRNA were only slightly increased in cell extracts prepared at 6 h postinfection compared with extracts prepared at 2 h postinfection, when mRNA degradation was negligible. The interferon-treated cells infected with reovirus, therefore, maintained a sustained rate of protein synthesis in spite of increased mRNA turnover.

It seemed possible that these cells could compensate for the loss of mRNA by enhanced production of new mRNA. We tested this hypothesis by treating the cells with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB). This drug specifically inhibits the synthesis of heterogeneous nuclear RNA at the initiation level, but does not interfere with the synthesis of rRNA or tRNA (5, 18). The DRB was added 90 min postinfection, and protein synthesis was moni-

TABLE 1. Protein synthesis in reovirus-infected cells[a]

Cell treatment	Reovirus added	DRB added	[^{3}H]lysine incorporation (cpm × 10^{-3}) at time postinfection: 3.5 h	5.5 h
Untreated	−	−	71.3	67.7
	+	−	67.7	69.4
	−	+	60.2	46.3
	+	+	57.8	45.6
Interferon	−	−	64.2	67.3
	+	−	61.7	62.8
	−	+	66.5	53.1
	+	+	61.3	27.2

[a] The cells were treated with 200 U of interferon per ml where indicated and infected or mock infected with reovirus at an MOI of 20; 0.1 mM DRB was added after 90 min, and samples of the cultures were incubated for 60 min with 10 μCi of [^{3}H]lysine per ml at 3.5 and 5.5 h after infection. Protein synthesis measurement at 1.5 h postinfection gave essentially identical values for all samples.

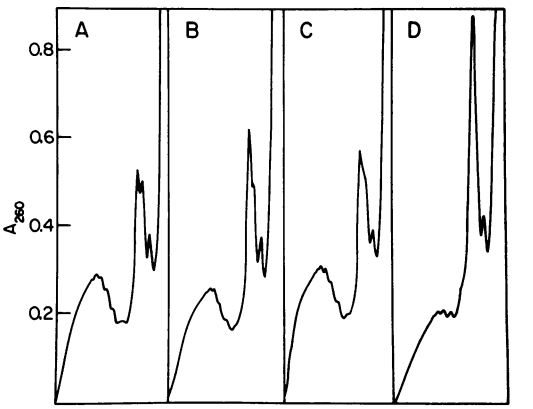

FIG. 3. Polysome profiles of interferon-treated cells infected with reovirus and treated with DRB (an inhibitor of RNA synthesis). (A and B) Control untreated cells; (C and D) interferon-treated cells; (A and C) mock infected; (B and D) infected with reovirus at an MOI of 20. To all cultures, 0.1 mM DRB was added 90 min after infection. The cells were harvested at 6 h postinfection, and cell extracts were prepared and analyzed as described in the text. A_{260}, Absorbance measured at 260 nm.

tored as described above. Protein synthesis was inhibited to some extent by DRB in uninfected cells and in infected cells not treated with interferon, but it was inhibited to a much greater extent in interferon-treated infected cells (Table 1). Inhibition of protein synthesis was confirmed by monitoring the polysome profile. The polysomes were sharply reduced in amount and the 80S ribosomes were correspondingly increased in interferon-treated cells infected with reovirus and treated with DRB (Fig. 3). These results were consistent with the hypothesis that synthesis of mRNA is necessary for the maintenance of a constant level of protein synthesis in these cells, since inhibition of mRNA synthesis resulted in loss of protein synthesis activity.

These experiments investigated the metabolism of all of the polyadenylate-containing mRNAs labeled with [^{3}H]uridine. To extend these observations to individual mRNAs, we fractionated RNA samples by gel electrophoresis and hybridized Northern blots to cloned cDNAs. Three different probes were used: the cloned cDNAs for the chicken α- and β-tubulins, which cross-hybridize to human tubulin mRNAs (4), and a cloned cDNA prepared from Chinese hamster ovary cell mRNA (8). The latter (clone B) cDNA was previously shown to cross-hybridize to HeLa cell mRNA (J. E. Darnell, personal communication). We used this nick-translated cDNA as a probe because it gave a strong signal on Northern blots (Fig. 4). The mRNAs hybridizing to these probes were present in about the same amount in both interferon-treated and untreated cells infected with reovirus, but in reduced amount in the latter cells incubated with DRB (Fig. 4). When RNA synthesis was inhibited by DRB, therefore, these cells could no longer compensate for its degradation and keep the mRNA level constant. It should be pointed out that in control cells treated with DRB there was no significant change in the amount of mRNAs hybridizing to the cloned cDNAs, indicating that there was relatively little mRNA turnover in cells incubated for 2 h with DRB only.

To establish whether some mRNAs were preferentially degraded in interferon-treated cells infected with reovirus, we examined the protein synthesized by these and by control untreated

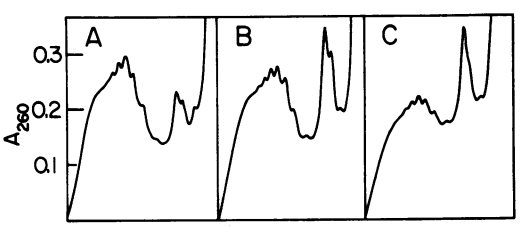

FIG. 2. Polysome profiles of interferon-treated cells infected with reovirus. Cells treated with interferon were infected at an MOI of 20. Culture samples were taken immediately before infection (A) and at 2 (B) and 6 h (C) after infection to prepare cell extracts, which were analyzed by centrifugation on sucrose gradients (see the text). A_{260}, Absorbance measured at 260 nm.

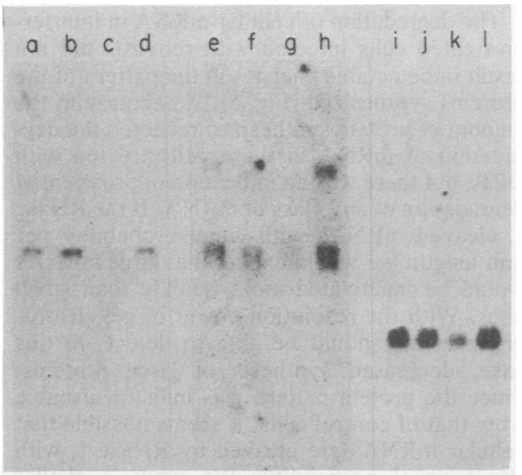

FIG. 4. Blot hybridization with cloned cDNAs to RNA prepared from (a, e, and i) mock-infected, interferon-treated cells, (b, f, and j) interferon-treated cells infected with reovirus, (c, g, and k) cells treated as for lanes b, f, and j, with 0.1 mM DRB added at 4 h postinfection, and (d, h, and l) untreated cells infected with reovirus. Total cellular RNA was prepared as described in the text at 6 h postinfection; 5 μg of RNA was fractionated on a 1.5% agarose gel and hybridized with nick-translated α-tubulin cDNA (a through d), β-tubulin cDNA (e through h), or clone B cDNA (i through l), as described in the text. The β-tubulin cDNA probe hybridizes to a major mRNA species, which migrates similarly to α-tubulin mRNA, and to a minor mRNA species, which migrates more slowly than the main mRNA species.

cells either infected with reovirus or mock infected, with or without added DRB (Fig. 5). These cells were incubated with [^{35}S]methionine from 5 to 6.5 h after infection or mock infection, and the labeled proteins were fractionated by gel electrophoresis. Protein synthesis in the interferon-treated cells infected with reovirus and incubated with DRB was 40% that of control cells, a value essentially identical to that obtained in labeling experiments with [^{3}H]lysine (Table 1). When the proteins were fractionated by gel electrophoresis, we could not detect any difference among the autoradiographic patterns, with the single exception of reovirus-infected cells not treated with interferon (note that in this analysis an identical amount of labeled protein was applied to each track). In this case an additional band was detected (Fig. 5) which could be a viral protein, since it was only seen in cells containing replicating reovirus. Therefore, it seemed unlikely that RNase L preferentially degraded some mRNA species, since there was no apparent difference between the proteins synthesized by interferon-treated cells infected with reovirus and by control cells.

DISCUSSION

The degradation of cellular mRNA in interferon-treated cells infected with reovirus did not result in detectable inhibition of protein synthesis. The ability of these cells to synthesize protein at a constant rate could be explained by the maintenance of the cytoplasmic concentration of mRNA via production of a compensatory amount of mRNA. This was indicated by the finding that protein synthesis declined when mRNA was degraded and synthesis of new mRNA was inhibited by DRB and was clearly established by measuring individual mRNA species by blot hybridization with specific cloned cDNAs. In interferon-treated cells infected with reovirus and incubated with DRB, the mRNA level was drastically reduced. These observations suggested that HeLa cells could increase the output of mRNA when the mRNA concentration became limiting for protein synthesis.

Hovanessian et al. (9) reported that protein synthesis is inhibited in tissue culture cells treated with 2′,5′-oligo(A) coprecipitated with calcium phosphate. The activation of RNase L in

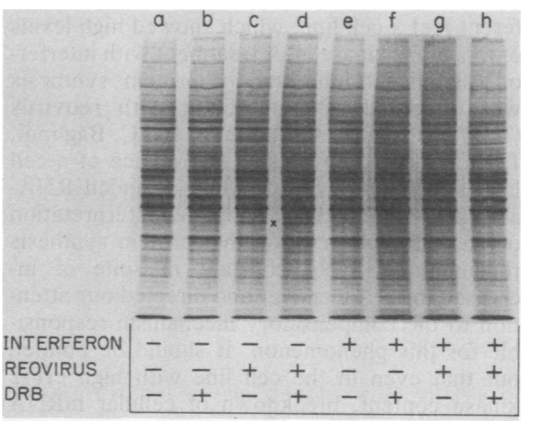

FIG. 5. Proteins synthesized in interferon-treated cells infected with reovirus. Cells treated with 200 U of interferon per ml (+) or untreated (−) were either mock infected (−) or infected with reovirus (+) at an MOI of 20. Where indicated, 0.1 mM DRB was added after 90 min. At 4.5 h the cells were collected by centrifugation and suspended in medium without methionine, with DRB again added where indicated. At 5 h the cells were labeled with [^{35}S]methionine for 90 min, and cytoplasmic extracts were prepared and fractionated by electrophoresis on 12.5% polyacrylamide gels as described by Laemmli (11). The amount of labeled protein synthesized was measured by counting an aliquot of each sample. The relative values obtained were essentially identical to those reported in Table 1. An identical amount of labeled protein (100,000 cpm) was applied for each sample. A band only seen in control cells infected with reovirus is indicated (X).

these cells results in breakdown of cellular RNA (9), and a transient inhibition of RNA synthesis (9) may prevent the cells from compensating for the loss of mRNA. Cells treated with 2',5'-oligo(A), however, return to a normal level of protein synthesis within a few hours, presumably by synthesizing new mRNA.

Interferon induces the synthesis of another enzyme which is also activated by double-stranded RNA, a protein kinase which phosphorylates the α subunit of initiation factor eIF-2 (1, 6). The activation of this eIF-2 kinase in cell-free protein synthesizing systems results in inhibition of protein synthesis (6). Since no inhibition of protein synthesis was observed in interferon-treated HeLa cells infected with reovirus, the cell line used in these experiments was examined for its content of double-stranded RNA-activated protein kinase with the assay previously described (20). These cells were found to have a rather low kinase content after treatment with interferon (16). The present experiments, therefore, were carried out in a HeLa cell line with a 2',5'-oligo(A) polymerase content enhanced up to 20-fold by treatment with interferon (13), but with a relatively low content of double-stranded RNA-activated kinase. In a different HeLa cell line, which showed high levels of this eIF-2 kinase after treatment with interferon, significant inhibition of protein synthesis was observed after infection with reovirus (T. W. Nilson, P. A. Maroney, and C. Baglioni, J. Biol. Chem., in press). The choice of a cell line with a low level of double-stranded RNA-activated protein kinase simplified interpretation of the initial observation that protein synthesis remained relatively constant in spite of increased mRNA turnover and directed our attention to the compensatory mechanism responsible for this phenomenon. It should be pointed out that even in the cell line with high eIF-2 kinase content, breakdown of cellular mRNA and synthesis of a compensatory amount of mRNA was observed.

This ability of HeLa cells to maintain a constant mRNA concentration in spite of ongoing mRNA degradation may represent a novel regulatory mechanism. Two ways of increasing mRNA production seem possible: (i) mRNA is transcribed at an enhanced rate, and (ii) a greater proportion of mRNA precursors are processed to mRNA. The present experiments cannot distinguish between these two pathways for increased mRNA production because DRB inhibits the synthesis of nuclear heterogeneous RNA and thus of mRNA. Future experiments directed at measuring the rate of synthesis of specific mRNAs and their corresponding nuclear precursors may clarify the molecular basis of this compensatory mechanism.

The degradation of cellular mRNA in interferon-treated cells infected with reovirus did not result in detectable changes in the pattern of the proteins synthesized (Fig. 5). A decrease in the amount of protein synthesized reflected the degradation of mRNA in these cells treated with DRB, but there was no indication of preferential degradation of any class of mRNA. If the RNase L cleaved mRNAs with equal probability per unit length, we would expect that large mRNAs would be inactivated more quickly than small ones. With the resolution power of gel electrophoresis we should be able to detect, in this case, decreased synthesis of large proteins. Since the protein pattern was indistinguishable from that of control cells, it seems possible that cellular mRNAs are cleaved by RNase L with about equal probability. We made a similar observation by examining the proteins synthesized by cells treated with 2',5'-oligo(A) coprecipitated with calcium phosphate. The mRNA was degraded and protein synthesis was drastically inhibited in these cells, but the same proteins were synthesized in these as in control untreated cells (unpublished data). Experiments are in progress to provide an explanation for this intriguing finding. In the present experiments, moreover, the pattern of proteins synthesized by cells not treated with DRB was indistinguishable from that in control cells. The interferon-treated cells infected with reovirus, therefore, not only maintain a constant rate of protein synthesis but continue to synthesize the same proteins as control cells. This can be accomplished either by producing additional mRNAs in a constant relative proportion or by regulating the rate of translation of different mRNAs. Although the latter explanation seems more likely, only further experiments may provide a definitive answer.

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LITERATURE CITED

1. **Baglioni, C.** 1979. Interferon induced enzymatic activities and their role in the antiviral state. Cell 17:255–264.
2. **Baglioni, C., M. A. Minks, and P. A. Maroney.** 1978. Interferon action may be mediated by activation of a nuclease by pppA2'p5'A2'p5'A. Nature (London) 273:684–687.
3. **Banerjee, A. K., and A. J. Shatkin.** 1970. Transcription in vitro by reovirus-associated ribonucleic acid-dependent polymerase. J. Virol. 6:1–11.
4. **Cleveland, D. W., M. A. Lopata, R. W. McDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α and β tubulin and cytoplasmic β and γ actin genes using specific cloned cDNA probes. Cell 20:95–105.

5. **Egyhazi, E.** 1975. Inhibition of Balbiani ring RNA synthesis at the initiation level. Proc. Natl. Acad. Sci. U.S.A. **72**:947–950.
6. **Farrell, P. J., K. Balkow, T. Hunt, and R. Jackson.** 1977. Phosphorylation of initiation factor eIF-2 and the control of reticulocyte protein synthesis. Cell **11**:187–200.
7. **Glisin, V., R. Crkvenjakov, and C. Byus.** 1974. Ribonucleic acid isolation by cesium chloride centrifugation. Biochemistry **13**:2633–2637.
8. **Harpold, M. M., R. M. Evans, M. Salditt-Georgiev, and J. E. Darnell.** 1979. Production of mRNA in Chinese hamster cells: relationship of the rate of synthesis to cytoplasmic concentration of nine specific mRNA sequences. Cell **17**:1025–1035.
9. **Hovanessian, A. G., J. Wood, E. Meurs, and L. Montagnier.** 1979. Increased nuclease activity in cells treated with pppA2'p5'A2'p5'A. Proc. Natl. Acad. Sci. U.S.A. **76**:3261–3265.
10. **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.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Maniatis, T., A. Keffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
13. **Minks, M. A., S. Benvin, P. A. Maroney, and C. Baglioni.** 1979. Synthesis of 2'5'-oligo(A) in extracts of interferon-treated HeLa cells. J. Biol. Chem. **254**:5058–5064.
14. **Montgomery, D. L., J. M. Boss, S. J. McAndrew, L. Marr, D. W. Walthall, and R. Zitomer.** 1982. The molecular characterization of three transcriptional mutations in yeast iso-2-cytochrome *c* gene. J. Biol. Chem. **257**:7756–7761.
15. **Nilsen, T. W., P. A. Maroney, and C. Baglioni.** 1982. Synthesis of (2'-5')oligo(A) and activation of an endoribonuclease in interferon-treated HeLa cells infected with reovirus. J. Virol. **42**:1039–1045.
16. **Ratner, L., R. C. Wiegand, P. J. Farrell, G. C. Sen, B. Cabrer, and P. Lengyel.** 1978. Interferon, double-stranded RNA and RNA degradation. Fractionation of the endonuclease$_{INT}$ system into two macromolecular components; role of a small molecule in nuclease activation. Biochem. Biophys. Res. Commun. **81**:947–954.
17. **Schmidt, A., A. Zilberstein, L. Shulman, P. Federman, H. Berissi, and M. Revel.** 1978. Interferon action: isolation of nuclease F, a translation inhibitor activated by interferon-induced (2'-5')oligo-isoadenylate. FEBS Lett. **95**:257–264.
18. **Sehgal, P. B., J. E. Darnell, Jr., and I. Tamm.** 1976. The inhibition by DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) of hnRNA and mRNA production in HeLa cells. Cell **9**:473–480.
19. **Thomas, P.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
20. **West, D. K., and C. Baglioni.** 1979. Induction by interferon in Hela cells of a double-stranded RNA activated protein kinase. Eur. J. Biochem. **101**:461–468.
21. **Williams, B. R. G., R. R. Golgher, R. E. Brown, C. S. Gilbert, and I. M. Kerr.** 1979. Natural occurrence of 2-5A in interferon-treated EMC virus-infected L cells. Nature (London) **282**:582–586.
22. **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)$_n$A) in interferon-treated cells. Nucleic Acids Res. **9**:1571–1581.