Structural requirements of (2'-5') oligoadenylate for protein synthesis inhibition in human fibroblasts

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ABSTRACT

The structural requirements of (2'-5')-oligoadenylic acid (pppA(2'p5'A)$_x$, $x \geq 1$ or (2'-5')A_n) for inhibition of protein synthesis in cells were examined with a modified calcium-coprecipitation technique, using a series of trinucleotide analogs (pppA2'p5'A2'p5'N, N = rC, rG, rU, T, dC, dG, dA). In this system, both the degree and the duration of the inhibition of protein synthesis were dependent on the added concentration of (2'-5')A_3. Of all the heterotrimers, only the deoxy A derivative was active as an inhibitor of protein synthesis, while the other members of the analog series were found to have no inhibitory effects. In competition experiments between (2'-5')A_3 and the non-active analogs, three heterotrimers were shown to reduce the activity of (2'-5')A_3 in protein inhibition. In contrast, the dephosphorylated (2'-5')A_3 had no inhibitory effect and was not effective in blocking (2'-5')A_3. These results indicate that the 5'-terminal triphosphate is important for binding of (2'-5')A_3 to the site of (2'-5')A_n action and the adenine base at the 2'-terminus is important for activating the machinery responsible for protein synthesis inhibition in the cells, most likely the (2'-5')A_n-activated nuclease.

INTRODUCTION

The (2'-5')-oligoadenylates pppA(2'p5'A)$_x$, $x \geq 1$ or (2'-5')A_n are produced from ATP in the presence of dsRNA by the (2'-5')A_n synthetase, an enzyme found in interferon (IFN) treated cells and rabbit reticulocyte lysates (1-3). Since the amount of (2'-5')A_n synthetase is enhanced in IFN-treated cells, the synthesis and action of (2'-5')A_n may play a major role in the interferon-induced effects in cells (4). In fact, the (2'-5')A_n in nanomolar concentrations is a potent inhibitor of protein synthesis in in vitro translation systems and in living mammalian cells (5-8).

These inhibitory effects of (2'-5')A_n on protein synthesis are exerted through the activation of a latent endoribonuclease present in interferon-treated and -untreated cells (9-15). Although the mechanism of endonuclease activation is not completely understood, it is clear that (i) activation of the endonuclease requires the binding of (2'-5')A_n to the enzyme (16); (ii)

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this activation results in the inhibition of protein synthesis by cleavage of single-stranded RNA, primarily at UA and UU sites (17,18); and (iii) the level of endonuclease activity and the resultant inhibition of protein synthesis in both cellular and cell-free systems are dependent on the concentration of added (2'-5')A_n. However, the potent effects of (2'-5')A_n are transient, presumably due to the rapid degradation of the oligonucleotides (19). The potent action and the short lifetime of the (2'-5')A_n are considered important features related to the specificity of the action of (2'-5')A_n (20).

In the present investigation, an effective and efficient system has been established for examining the effects of (2'-5')A_n on protein synthesis in human fibroblast cells in culture. Using a modified $CaCl_2$ coprecipitation technique similar to that of Hovanessian et al., with a suitable carrier, the system was employed to determine the structural requirements for the inhibitory action of (2'-5')A_n on protein synthesis in living cells, presumbly via the activation of the endonuclease (8).

MATERIALS AND METHODS

Materials. All radioactive compounds were purchased from New England Nuclear Corporation. Poly I·poly C-agarose, bacterial alkaline phosphatase, polyA, and (3'-5')ApApA were obtained from P-L Biochemicals.

Preparation of (2'-5')A_n and Hetero-oligonucleotide Analogs. The enzymic synthesis of (2'-5')A_n and the purification of specific (2'-5') oligomers by DEAE-sepharose chromatography were performed as described earlier (21). The purity of each size class in the (2'-5')A_n series was checked using PEI-cellulose chromatography as previously described (21), and by high performance liquid chromatography; no contaminants could be detected except for the dimer that contained 10% ATP. Dephosphorylated (2'-5')A_n was also checked by HPLC and found to contain the same concentrations of dimer, trimer, tetramer and pentamer as the starting (2'-5')A_n solution. Here, (2'-5')A_n refers to the group of oligonucleotides (pppA(2'p5'A)$_x$, $x > 1$). The purified oligonucleotides are defined as: dimer, (2'-5')A_2; trimer, (2'-5')A_3; tetramer, (2'-5')A_4; and pentamer, (2'-5')A_5. Concentration of (2'-5')A_n was spectrophotometrically determined using an extinction coefficient of 15.4 mM.cm for AMP. The heterotrimers pppApApN (N = rC, rG, rU, T, dG, dC, dA) were synthesized by incubation of a 1mM (2'-5')A_2 with excess nucleoside triphosphate and (2'-5')A_n synthetase (21). Under the conditions used, 100% of the dimer was converted to hetero-oligonucleotide trimer (22).

Cell Growth. The growth and propagation of human cell strain HF926 has been described previously (23). For this study 2.5 x 10^4 cells per well were plated into 96-well cluster plates (Costar) at least 24 hours prior to use.

(2'-5')A_n Treatment of Cells. A slight modification of the calcium phosphate coprecipitation procedure of Hovanessian et al. (8) was used to introduce the (2'-5')A_n into the intact cells. For treatment, growth medium in each well was replaced with 50 µl of Hepes-buffered saline (HBS) (pH 7.05) (8 g of NaCl, 0.37 g of KCl, 1.25 g of $Na_2HPO_4 \cdot 2H_2O$, 1.0 g glucose, and 5.0 g Hepes per liter) containing 125 mM $CaCl_2$, carrier (5 x 10^{-5}M, consisting of short (3'-5') linked oligo A's produced by alkaline hydrolysis of poly A) and (2'-5')$_n$A at a desired concentration indicated in each experiment. After 1 hour at room temperature, all samples, except where noted, were refed with 200 µl of medium (ERM-met 10) containing 1.5 µg/ml methionine and then were incubated for an additional hour at 37°C prior to labeling.

Certain samples were used to measure long-term effects of (2'-5')A_n and the recovery of protein synthesis following (2'-5')A_n treatment. These samples were either washed three times and refed (washed set) or directly refed with 200 µl of media + 10% serum (ERM and 10% serum). All cells were refed with ERM-met 10 one hour prior to labeling.

Measurement of Cellular Protein Synthesis. To label the cells, each well was refed with ERM-met 10 containing 10% dialyzed fetal calf serum and [^{35}S]-methionine (1000 Ci/mmol, 4 µCi well) for 60 minutes or as indicated otherwise. The plates were then quickly washed with phosphate buffered saline and lysed with 10% SDS. The lysate was precipitated in 10% TCA in the presence of 100 µg/ml bovine serum albumin at 70°C for 30 minutes. Acid insoluble material was collected on Millipore filters and the radioactivity was measured after dissolution of the filter in 10 ml of Betafluor (National Diagnostics).

RESULTS

(3'-5')A carrier enhances the inhibitory effect of (2'-5')A_n on protein synthesis. The human cell strain HF926 treated with exogenous (2'-5')A_3 through $CaCl_2$ precipitation was found to exhibit a dramatic decrease in protein synthesis as measured by incorporation of [^{35}S]-methionine into acid insoluble material, similar to that previously shown by Hovanessian et al. (8).

For an efficient uptake of (2'-5')A_n by the cells, the formation of a $CaCl_2$-oligo A coprecipitate is required, presumably in a manner analogous to the $CaCl_2$ precipitation-mediated uptake of DNA and RNA (24, 25). The addition of inert carrier which consists of short (3'-5')-linked oligo A was found to

improve significantly the efficiency of the (2'-5')A_3 as a protein synthesis inhibitor (Figure 1). Application of a (3'-5')A_3 precipitated by $CaCl_2$, as well as the mere addition of $CaCl_2$ alone, had no effect on protein synthesis as measured by [^{35}S]-methionine incorporation.

This increase in effectiveness of protein synthesis inhibition by (2'-5')A_3 reflects the effect of the carrier. Measurements of the degree of $CaCl_2$ precipitation of [^{3}H]-or [^{14}C]-(2'-5')A_2 and (2'-5')A_3 showed that (2'-5')A_2 was precipitated to a lesser extent than (2'-5')A_3. In addition, higher concentrations of each oligomer were precipitated more efficiently than lower concentrations. However, the addition of carrier to the radioactive oligomers equalized the percentage of precipitation in all these samples regardless of the chain length or concentration of the oligomer. It should be noted, however, that the inhibition of protein synthesis by (2'-5')A_3 is directly dose-dependent on (2'-5')A_3 with or without the carriers, although the use of the carrier improved significantly both the extent and reproducibility of the (2'-5')A_3 effects. This great enhancement of effectiveness in the assay of cellular protein synthesis inhibition allows a more accurate determination of the biological effects of purified (2'-5')A_3 and its analogs over the entire

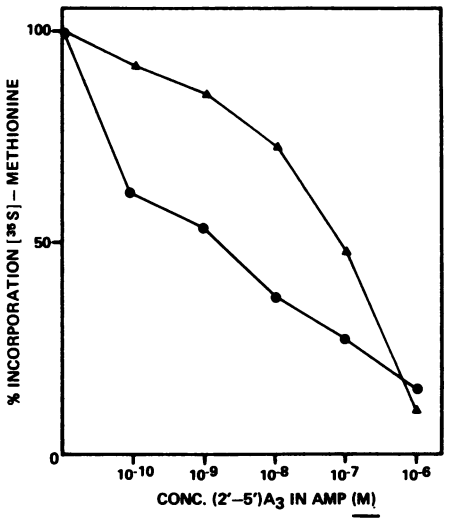

FIGURE 1. The effect of carrier on (2'-5')A_n action. Cells were incubated with HBS containing $CaCl_2$ (125 mM), (2'-5')A_3 with carrier (●——●), 5 x 10^{-5}M) and without carrier (▲——▲) for one hr at room temperature. All wells were then labeled as in materials and methods. The HBS + $CaCl_2$ alone contained 24,200 CPM and the HBS + $CaCl_2$ + carrier had 23,400 CPM.

concentration range.

It is known that $(2'-5')A_n$ is rapidly degraded in cell extracts (19). However, the fate of $(2'-5')A_n$ inside living cells is unclear. One way to provide some information on this question is to determine whether the inhibition of cellular protein synthesis exerted by the exogenous $(2'-5')A_n$ is transitory. Hovanessian and Wood examined the effect of $CaCl_2$-precipitate $(2'-5')A_3$ on protein synthesis in Mouse L 929 cells and found that inhibition of protein synthesis was transient at a low concentration but persistent at higher $(2'-5')A_3$ concentrations (9). Using a hypertonic shock technique which allows for the uptake of molecules from the medium for a brief period of time, Williams and Kerr showed that protein synthesis inhibition in BHK cells following a short exposure to exogenous $(2'-5')A_3$ lasted only 3 hrs. (26). Since cells continue to take up material from $CaCl_2$ coprecipitates as long as the precipitate is attached to the cell (unlike hypertonic shock) we have examined the duration of protein synthesis inhibition in human cells treated with $(2'-5')A_3$ by calcium precipitation. Following treatment with $(2'-5')A_3$ one group of cultures was refed directly (unwashed set) and one group of cultures was washed extensively to remove calcium precipitate prior to refeeding (washed set). In both sets, the appearance of protein synthesis inhibiton was rapid; however, recovery of protein synthesis became noticeable within 8 hrs in the washed set. By 24 hrs, protein synthesis in the washed set returned to near normal levels and mitotic figures were seen in the sample. When cultures from the unwashed set were washed 3 hours after treatment with $(2'-5')A_3$, protein synthesis levels recovered significantly within 24 hrs. When the culture remained in the unwashed condition for 24 hrs, protein synthesis remained completely inhibited. These results indicate that a prolonged maintenance of protein synthesis inhibition requires a constant supply of $(2'-5')A_3$; a 3 hour treatment with $(2'-5')A_3$ does not produce lasting damage to the cell as determined by recovery of protein synthesis. The observed rate of recovery of protein synthesis may reflect a combination of the rate of intracellular breakdown of $(2'-5')A_3$ and the re-establishment of mRNA levels required for growth.

Structural Requirements of $(2'-5')A_n$ for Inhibition of Protein Synthesis. Figure 2 depicts the comparative activities of $(2'-5')A_n$ versus the dephosphorylated $(2'-5')A_n$ core which was obtained by extensive treatment with bacterial alkaline phosphatase. The dephosphorylated $(2'-5')A_n$ core was relatively inactive even after long incubation times, indicating that little or no rephosphorylation of the dephosphorylated $(2'-5')A_n$ occurs in these cells.

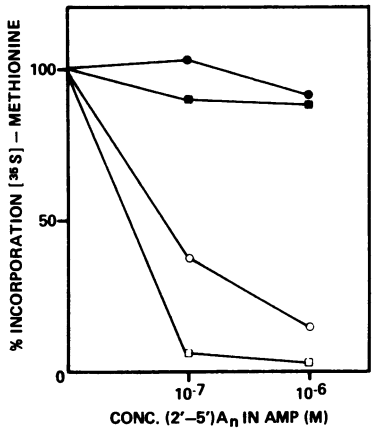

FIGURE 2. Protein synthesis inhibition by (2'-5')A_n and dephosphorylated (2'-5')A_n. To determine the effect of dephosphorylated (2'-5')A_n in contrast to (2'-5')A_n on protein synthesis, cultures were treated with dephosphorylated (2'-5')A_n for 2 hrs (● —●) or 8 hrs (■—■); with (2'-5')A for 2 hrs (○—○) or 8 hrs (□—□). The samples were treated for 1 hr at room temperature in HBS containing 125mM $CaCl_2$ at the concentrations of oligonucleotide indicated, then directly fed with ERM + 10% FCS. One hr prior to labeling, the samples were refed with ERM-met 10. Cells were then labeled with [^{35}S]-methionine.

Measurement of $CaCl_2$ precipitation of [^{3}H]-or[^{14}C]-(2'-5')A_n or dephosphorylated (2'-5')A_n showed that each sample was precipitated to the same extent. The lack of inhibitory activity of the dephosphorylated (2'-5')A_n was therefore not a problem of precipitation but more likely due to a failure to rephosphorylate the (2'-5')A_n. The failure to rephosphorylate the (2'-5')A_n appears to be unique to this system. Williams and Kerr and Kimchi and Revel have shown that the core is active in their respective systems (7, 27, 28). The requirement of 5' terminal triphosphate for endonuclease binding and activation has been demonstrated in cell free systems (16,30).

Recently, it has been shown that (2'-5')A_n synthetase can catalyze the formation of a series of oligonucleotides of structure pppA(2'p5'A)$_n$2'p5'N (N = rC, rG, rU, T, dC, dG, and dA) (16, 31). A series of heterotrimers was prepared for comparative studies with trimer. Table 1 shows the results on protein synthesis inhibition obtained with two different concentrations of heterotrimers in direct comparison with (2'-5')A_3. Of all the heterotrimers tested, only the (2'-5')A_2dA showed consistent biological activity in inhibition of cellular protein synthesis. The biological activity of

(2'-5')A_2dA trimer directly parallels the activity of (2'-5')A_3 in dose response and magnitude (Table 1).

The results suggest a specific requirement for adenine at the 2' terminus of the trimer, the dA being as effective as the rA for protein synthesis inhibition and presumably endonuclease activation. This result supports the conclusion of Doetsch et al. (32, 33) and Baglioni et al. (30) that the 2' and 3' hydroxyl groups at the 2'-terminus can be modified without causing a reduction in biological activity of (2'-5')A_3.

The failure of the other heterotrimers to inhibit cellular protein synthesis does not necessarily indicate that these oligomers cannot interact

Table 1. Effect of (2'-5')A_n and (2'-5')A_2N on Protein Synthesis

Oligonucleotide	% [^{35}S]-Methionine Incorporation			
Purified (2'-5')A_n	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M
(2'-5')A_2	90	82	81	62
(2'-5')A_3	54	40	28	12.5
(2'-5')A_4	50	44	33	15
(2'-5')A_5	52	41	33	25

(2'-5')-Trimers	10^{-8}M	2 x 10-7M	10^{-6}M
(2'-5')A_3	40	25.5	12.5
(2'-5')A_2G	104	107	-
(2'-5')A_2C	100	101	-
(2'-5')A_2U	97	100	-
(2'-5')A_2T	105	106	-
(2'-5')A_2dG	96	91	-
(2'-5')A_2dA	37	27	17

Cells were incubated with HBS containing $CaCl_2$ (125 mM), carrier (5×10^{-5}M) and the compound at the concentration indicated.
After treatment for 1 hr, the plates were fed with ERM-met 10 and incubated an additional hr. Cells were then labeled by refeeding with ERM-met 10, 20% FCS, plus ^{35}[S]-methionine. After 1 hr of labeling, the cells were processed as described. The untreated control sample (HBS + Carrier + $CaCl_2$) had 35,900 CPM, and was considered to be 100%

with the site of $(2'-5')A_n$ action. As shown in Table 2, the simultaneous application of both $(2'-5')A_3$ and the heterotrimer, results in a noticeable reduction in the inhibition caused by $(2'-5')A_3$. Here again, the antagonistic effect of the heterotrimers in the inhibition of protein synthesis caused by $(2'-5')A_3$ is proportional to the concentrations of the analog as illustrated by the effects of three concentrations of $(2'-5')A_2C$.

This competition effect does not appear to be due to a dilution of the

Table 2. Effect of $(2'-5')A_2N$ on $(2'-5')A_3$ Protein Synthesis Inhibition

Sample	Incorporation CPM	% Inhibition	% of $(2'-5')A_3$ Inhibitory Activity Remaining
A. Competition			
Control	33,700	-	-
$(2'-5')A_3$	13,700	59	100
$(2'-5')A_3$ + $(2'-5')A_2G$	23,200	31	53
$(2'-5')A_3$ + $(2'-5')A_2U$	16,600	50	86
$(2'-5')A_3$ + $(2'-5')A_2T$	22,400	33	57
$(2'-5')A_3$ + dephosphorylated $(2'-5')A_n$	15,200	54	93
$(2'-5')A_3$ + dephosphorylated $(2'-5')A_n$ (1×10^{-8}M)	18,000	46	79
B. Dose Response with $(2'-5')A_2C$		% Inhibition	% of $(2'-5')A_3$ Activity Remaining
		Exp. I,II	Exp. I,II
$(2'-5')A_3$		73, 74	100,100
$(2'-5')A_3$ + $(2'-5')A_2C$ (2×10^{-8}M)		60, 59	81, 80
$(2'-5')A_3$ + $(2'-5')A_2C$ (2×10^{-7}M)		54, 41	72, 55
$(2'-5')A_3$ + $(2'-5')A_2C$ (2×10^{-6}M)		23, 29	31, 39

Effect of $(2'-5')A_2N$ on $(2'-5')A_3$-mediated cellular protein synthesis inhibition. The heterotrimers or dephosphorylated $(2'-5')A_n$ (2×10^{-7}M, unless specified) were mixed with carrier (5×10^{-5}M) and trimer (1×10^{-8}M) prior to precipitation with $CaCl_2$ (125 mM). The mixture was applied and the plates were incubated 1 hr at room temperature. The plates were then directly fed with ERM-met 10, and labeled 1 hr later. The percent inhibition of protein synthesis reflects the overall inhibitory activity of a particular sample. The percent of $(2'-5')A_3$ inhibitory activity reflects the remaining activity of $(2'-5')A_3$ within a sample versus the treatment with $(2'-5')A_3$ alone. The second part of the table shows results of a separate experiment on the dose-response of the blocking effect of a heterotrimer on $(2'-5')A_3$.

(2'-5')A_3 because the presence of dephosphorylated (2'-5')A_n has virtually no effect on the action of (2'-5')A_3 (Table 2), or by a change in the $CaCl_2$ precipitation of the trimer. This type of competitive effect is similar to that reported recently by Torrence et al. (34) who showed that monophosphorylated (2'-5')A_3 blocks (2'-5')A_3 mediated protein synthesis inhibition and messenger RNA degradation in vitro. Monophosphorylated (2'-5')A_3 has been shown previously to interact with the endonuclease in vitro (16). As with the monophosphorylated (2'-5')A_3, perhaps the (2'-5') heterotrimers are capable of interacting with the (2'-5')A_n-dependent endonuclease without activating the enzyme.

DISCUSSION

In order to evaluate the effects of exogenous (2'-5')A_n on living cells, a hypertonic swelling technique (7,26) and a $CaCl_2$ precipitation procedure (8,9) were used previously to facilitate the cellular uptake of (2'-5')A_n. These studies have demonstrated the inhibition of protein synthesis and enhancement of endonuclease activity in (2'-5')A_n-treated cells. In this investigation, addition of (3'-5') oligoadenylate as carrier has greatly improved the efficiency of the inhibitory action of (2'-5')A_n at dilute concentrations. This improvement results from the presence of a large excess of inactive carrier causing a uniform coprecipitation of all oligonucleotides and possibly providing the protection of (2'-5')A_n from enzymatic degradation. This procedure provides a more defined system for the determination of the structural requirements of the (2'-5')A_n, by eliminating the possible influence of oligonucleotide concentration, or of any other factor which may influence the coprecipitation process.

With this improved assay on human fibroblasts, the structural requirements for (2'-5')A_n-mediated protein synthesis inhibition were examined leading to the following three conclusions: First, in agreement with the findings of Williams and Kerr (7) and Knight et al. (16) the minimum chain length for (2'-5')A_n has been found to be three nucleotides (Table 1). Second, in this system the 5'-terminal triphosphate is required. Third, the presence of an adenine base at the 2'-terminus is essential for biological activity of the trimer. With regard to the second conclusion, Baglioni et al. (30) has shown that a terminal diphosphate (2'-5')A_3 is almost as active as a triphosphate (2'-5')A_n (25). Williams and Kerr have reported that dephosphorylated (2'-5')A_3 inhibited protein synthesis in whole cells. This discrepancy could have arisen in the following ways. The hypertonic shock technique requires the addition of large

amounts of high energy phosphate (1mM ATP, 0.1mM GTP, and 100mM creatine phosphate) to stimulate recovery of the cells. Perhaps this excess ATP can stimulate rephosphorylation of the (2'-5')A_n core. Another possibility is that the core (2'-5')A_3 is degraded before it is rephosphorylated in HF926. Baglioni et al. has recently shown that monophosphorylated-mono-3'-O-CH_3-(2'-5')A_3 became biologically active in human cell extracts via rephosphorylation. However, core-mono-3'-O-CH_3(2'-5')A_3 without the α-5'-phosphate was not rephosphorylated or active (30). With regard to the third conclusion, this is the first study to examine the effect of base composition on (2'-5')A_3 function. Knight et al. has shown that the tetramer pppA2'p5'A2'p5'A3'p5'Cp binds to the endonuclease and inhibits protein synthesis in vitro (16). This observation suggests that the endonuclease may only recognize the first three nucleotides. While the heterotrimers described in this study could not inhibit protein synthesis, they were able to act as competitors to (2'-5')A_3, in reducing protein synthesis inhibition. Since these compounds have this unique ability to compete with (2'-5')A_3 and since they can be produced enzymatically, two questions therefore should be raised. Are these heterotrimers produced normally in the cell? If so, what are the functions of these oligonucleotides? The (2'-5')-linked hetero-oligonucleotides, as well as the other products of the (2'-5')A_n synthetase described by Februs et al. (26) and Ball (36) could play other roles besides endonuclease activation important to cellular function.

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REFERENCES

1. Hovanessian, A.G. and Kerr, I.M. (1978) Eur. J. Biochem. 84, 149-159.
2. Farrel, P.J., Sen, G.C., Dubois, M.F., Ratner, L., Slattery, E. and

Lengyel, P. (1978) Proc. Natl. Acad. Sci. USA 75, 5893-5897.
3. Ball, L.A. (1979) Virology 94, 282-296.
4. Baglioni, C. (1979) Cell 17, 255-264.
5. Kerr, I.M. and Brown, R.E. (1978) Proc. Natl. Acad. Sci. USA 75, 256-260.
6. Ball, L.A. and White, C.N. (1978) Proc. Natl. Acad. Sci. USA 75, 1167-1171.
7. Williams, B.R.G. and Kerr, I.M. (1978) Nature 276, 88-90.
8. Hovanessian, A.G., Wood, J., Meurs, E. and Montagnier, L. (1979) Proc. Natl. Acad. Sci. USA 76, 3261-3265.
9. Hovanessian, A.G. and Wood, J.N. (1980) Virology 101, 81-90.
10. Ratner, L., Sen, G.C., Brown, G.E., Lebleu, B., Kawakita, M., Cabrer, B., Slattery, E. and Lengyel, P. (1977) Eur. J. Biochem. 79, 565-577.
11. Clemens, M.J. and Williams, B.R.G. (1978) Cell 13, 565-572.
12. Baglioni, C., Minks, M.A. and Maroney, P.A. (1978) Nature 279, 684-687.
13. Schmidt, A., Zilberstein, A., Shulman, L., Federman, P., Berissi, H. and Revel, M. (1978) FEBS Lett. 95, 257-264.
14. Rattner, L., Wiegand, R.C., Farrell, P.J., Sen, G.C., Cabrer, B., and Lengyel, P. (1978) Biochem. Biophys. Res. Commun 81, 947-954.
15. Eppstein, D.A. and Samuel, C.E. (1978) Virology 89, 240-251.
16. Knight, M., Cayley, P.J., Silverman, R.H., Wreschner, D.H., Gilbert, C.S., Brown, R.E. and Kerr, I.M. (1981) Nature 288, 189-192.
17. Wreschner, D.H., McCauley, J.W., Skehel, J.J. and Kerr, I.M. (1981) Nature 289, 414-417.
18. Floyd-Smith, G., Slattery, E. and Lengyel, P. (1981) Science 212, 1030-1032.
19. Minks, M.A., Benvin, S., Maroney, P.A. and Baglioni, C. (1979) Nucl. Acids Res. 6, 767-780.
20. Nilsen, T.W. and Baglioni, C. (1979) Proc. Natl. Acad. Sci. USA 76, 2600-2604.
21. Justesen, J., Ferbus, D. and Thang, M.N. (1980) Nucl. Acids Res. 8, 3073-3085.
22. Justesen, J., Ferbus, D. and Thang, M.N. (1980) Proc. Natl. Acad. Sci. USA 77, 4618-4622.
23. Greene, J.J., Dieffenbach, C.W., Yang, L.C. and Ts'o, P.O.P. (1980) Biochem. 19, 2485-2491.
24. Graham, F.L. and Van Der Eb, A.J. (1973) Virology 52, 456-467.
25. Greene, J.J., Dieffenbach, C.W. and Ts'o, P.O.P. (1979) Nature 271, 81-83.
26. Williams, B.R.G., Golgher, R.R. and Kerr, I.M. (1979) FEBS Lett. 105, 47-52.
27. Kimchi, A., Shure, H. and Revel, M. (1979) Nature 282, 849-851.
28. Kimchni, A. and Revel, M.N., personal communication.
29. Martin, M., Birdsall, N.J.M., Brown, R.E. and Kerr, I.M. (1979) Eur. J. Biochem. 95, 295-307.
30. Baglioni, C.,D'Alessandro, S.B., Nilsen, T.W., Den Hartog, J.A.J., Grea, R. and van Boom, J.H. (1981) J. Biol. Chem. 256, 3253-3257.
31. Ferbus, D., Justesen, J., Besancon, F. and Thang, M.N. (1981) Biochem. Biophys. Res. Comm. 100, No. 2, 847-856.
32. Doetsch, P., Wu, J.M., Sawada, Y., Suhadolnik, R.J. (1981) Nature 291, 355-358.
33. Doetsch, P., Suhadolnik, R.J., Sawada, Y., Mosca, J.D., Flick, M.B., Reichenbach, N.L., Dang, A.Q., Wu, J.M., Charubala, R., Pfleiderer, W., and Henderson, E.E. (1981) Proc. Natl. Acad. Sci. USA 78, 6699-6703.
34. Torrence, P.L., Imia, J., Johnston, M.I. (1981) Proc. Natl. Acad. Sci. USA 78, 5993-5997.

35. Wreschner, D.H., James, T.C., Silverman, R.H. and Kerr, I.M. (1981) Nucl. Acids Res. 9, 1571-1581.
36. Ball, L.A. (1980) Annals of New York Acad. of Sci. 350, 486-496.