Synthesis of (2'-5')(A)n, from ATP
CHARACTERISTICS OF THE REACTION CATALYZED BY (2'-5')(A)n, SYNTHETASE PURIFIED FROM MOUSE EHRlich ASCITES TUMOR CELLS TREATED WITH INTERFERON*

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The treatment of Ehrlich ascites tumor cells with mouse interferon increases the level of the latent enzyme (2'-5')(A)n synthetase. If activated by double-stranded RNA, this catalyzes the synthesis from ATP of a series of 2'-5'-oligoadenylates: (2'-5')(A)n, where n extends from 2 to about 15. We isolated (2'-5')(A)n synthetase in a homogeneous state. In the presence of double-stranded RNA, the purified enzyme can convert the large majority (about 97%) of the ATP into (2'-5')(A)n, and pyrophosphate, although it does not cleave the pyrophosphate. The stoichiometry of the reaction can be formulated as: (n + 1) ATP → (2'-5')pppA(pA)n + n pyrophosphate. Added pyrophosphate does not inhibit the synthesis of (2'-5')(A)n. The extent of the reverse reaction, i.e., the pyrophosphorolysis of (2'-5')(A)n, was below the level of detection under our conditions. The affinity of the enzyme for ATP is low: the concentration of ATP is increased from 5 mm to 10 mm. The optimal concentration of double-stranded RNA increases with the concentration of the enzyme. As tested at 0.4, 2, and 10 μg/ml of enzyme concentrations, close to maximal (2'-5')(A)n, synthesis can be obtained if reovirus double-stranded RNA or poly(I)-poly(C) are used at about half the concentration (in w/v) of the enzyme. The plot of the reaction rate versus enzyme concentration is sigmoidal. It remains to be seen if this reflects on a cooperative behavior of the enzyme.

Interferons are glycoproteins synthesized by various kinds of vertebrate cells upon viral infection or some other stimuli. They are released from the producing cells, interact with other cells, and alter the biochemical and immunological characteristics of these (1, 2). The treatment of cells with interferon also results in the enhanced accumulation of certain messenger RNAs and proteins (3-7) and an increase in the level of various enzyme activities. Some of these enzymes (e.g., a protein kinase and an endonuclease system) are latent unless activated by double-stranded (ds)RNA and ATP (8-16).

Earlier, the endonuclease was divided into two complementory fractions. One of these fractions when incubated with dsRNA and ATP was shown to give rise to a small thermostable product. This in turn was found to activate a latent endonuclease, now designated as RNase L, in the other complementary fraction (17, see also Refs. 18 and 19). The small thermostable product was identified as (2'-5')(A)n, a series of compounds originally discovered as inhibitors of protein synthesis that are formed from ATP in extracts of interferon-treated cells in the presence of dsRNA (20). (2'-5')(A)n was detected in interferon-treated virus-infected cells (21). (2'-5')(A)n, has also been introduced into cells and was shown to cause a transient nuclease activation and an inhibition of protein synthesis and virus replication (22-24). (2'-5')(A)n, introduced into lymphocytes was found to impair mitogen-induced DNA synthesis (25).

(2'-5')(A)n synthetase has been detected in Ehrlich ascites tumor cells, HeLa cells, and chick embryonic fibroblasts treated with interferon as well as in mouse lymphocytes and rabbit reticulocytes, even if not previously exposed to interferon (17, 18, 26-28). The level of the enzyme was reported to increase in chicken oviducts after withdrawal of estrogens (29). The enzyme from chick embryonic fibroblasts has been partially purified by conventional procedures (16) and the enzyme from L cells by affinity chromatography on dsRNA (10).

The synthesis of (2'-5')(A)n proceeds in the 5' to 2' direction (16). The activated enzyme can use short 2'-5'-oligoadenylates, NAD, or diribonucleoside monophosphates containing a 3'-adenosine residue or AppppA as primers (16). dsRNA (poly(I)-poly(C) or poly(A)-poly(U)) shorter than 30 base pairs fails to activate the enzyme, whereas dsRNA longer than 65 to 80 base pairs causes maximal activation. The partially double-stranded polymers poly(I)-poly(G,C) with an average of one mismatch for every eight nucleotides do not activate the enzyme, whereas those with an average of one mismatch for every 45 nucleotides do activate it (30).

We have described earlier the isolation of pure (2'-5')(A)n synthetase from EAT cells treated with interferon. As determined by polyacrylamide gel electrophoresis in the presence of SDS, the apparent molecular weight of the enzyme was around 105,000. The activity of the enzyme was enhanced by dsRNA at least 25-fold. The size distribution of the (2'-5')(A)n, synthesized extended from the dimer to the pentadecamer (31). Here we report on further characteristics of the purified enzyme, and of the reaction it catalyzes.

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† The abbreviations used are: ds, double-stranded; A, 2'- or 3'-deoxyadenosine moiety; 2'Ap, 2'-adenylate; 3'Ap, 3'-adenylate; (2'-5')(A)n, 2'-5'-linked pppA(pA)n, where n is between 2 and 15; EAT, Ehrlich ascites tumor; PEI, polyethylenimine; Pipes, 1,4-piperazinediethanesulfonic acid; SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazinediethanesulfonic acid.

It should be noted that pppA(pA)n, and (2'-5')(A)n, stand for the same compound. The formula pppA(pA)n, is used when the emphasis is on the 5'-terminal triphosphate moiety of the compound, and (2'-5')(A)n, is used in other cases. We use n (to indicate chain length) in both cases to avoid unnecessary complication. In precise terms, however, (2'-5')(A)n, will correspond to pppA(pA)n,.
The purification of (2'-5')<sub>A</sub> synthesis from the low speed supernatant fraction from EAT cells which had been treated with 500 mM glucose reference standard units of interferon-alpha by differential precipitation with ammonium sulfate and chromatography on DEAE cellulose and CM cellulose was performed according to published procedures (31). The enzyme was assayed according to the "in solution assay" (31). The standard reaction mixture included 30 mM Hepes KOH (pH 7.8), 125 mM KCl, 8 mM Mg(CH3COO)2, 5 μg/μl poly(I)-poly(C), 1 μM α-32P ATP, 3 μM (O-A)₉ and no enzyme (a) or 0.78 nM (O-A)₉ enzyme or no enzyme (b) and 0.18 nM Trition X-100 (c), or no on Trition X-100 (D, A, O). Incubation was for the times indicated. ATP polymerized (mmol D, A, O), ATP polymerized (e), ATP remaining (l a). The data points connected with continuous lines are from a different experiment from those connected with discontinuous lines.

To avoid overlapping of data the scale on the ordinate for the data points connected with discontinuous lines is displaced from the scale for the data points connected with continuous lines.

RESULTS

Kinetics of (2'-5')<sub>A</sub> Synthesis: Optimization of Reaction Condition—The curves in Fig. 1 indicate that under the conditions of the incubation the rate of (2'-5')<sub>A</sub> synthesis decreases with time. This is apparently in consequence of the inactivation of the purified enzyme used at low concentrations.

The inactivation cannot be overcome by adding bovine serum albumin (1 mg/ml) to the reaction mixture and it also occurs when the enzyme is incubated without ATP (not shown).

In consequence of the inactivation of the enzyme during the incubation and the need for the formation of sufficient amounts of (2'-5')<sub>A</sub> for precise assay, we optimized the composition of the reaction mixture for the following conditions: synthesis from 1 mM ATP with 2 μg/ml of enzyme at 30°C for 2 h. For this optimization, we started with the assay conditions used in the purification of the enzyme and varied

\[\text{conditions of the incubation the rate of (2'-5')(A),, synthesis is close to optimal if the concentration of poly(I)-poly(C) or}\]

the concentration of the various components of the reaction mixtures one by one.

We found that the optimal concentration of KCl is 125 mM. The replacement of KCl by equimolar KOAc results in about an 18% decrease in (2'-5')(A), synthesis. However, with KOAc at its optimal concentration (225 mM), the yield of (2'-5')(A), is about 25% higher than with KCl at its optimal concentration (not shown). The (2'-5')(A), synthesis versus pH curve shows a peak at about pH 7.8 when determined in the presence of either KOAc or KCl (Fig. 2). The optimal temperature of the reaction is 25°C; the yield of (2'-5')(A), is 10% lower at 30°C (which is close to room temperature), unless otherwise specified we performed the experiments at 30°C.

When assayed at 1 mM ATP concentration, the optimal Mg acetate concentration is 8 mM whether tested at 30°C (Fig. 3A) or 25°C (not shown). An increase in the concentration of ATP necessitates a similar increase in Mg<sup>2+</sup> concentration to keep the conditions optimal (Fig. 3A). The data points connected with continuous lines are from a different experiment from those connected with discontinuous lines. The reaction mixture one by one.

The affinity of the enzyme for ATP polymerized (mmol D, A, O), ATP polymerized (e), ATP remaining (l a).

The data points connected with continuous lines are from a different experiment from those connected with discontinuous lines.

When assayed at 1 mM ATP concentration, the optimal Mg acetate concentration is 8 mM whether tested at 30°C (Fig. 3A) or 25°C (not shown). An increase in the concentration of ATP necessitates a similar increase in Mg<sup>2+</sup> concentration to keep the conditions optimal (Fig. 3A). Thus, in the presence of 5 mM ATP, the optimal Mg<sup>2+</sup> concentration is 12 mM and, at 10 mM ATP, the optimal Mg<sup>2+</sup> concentration is 16 mM.

The affinity of the enzyme for ATP is low; (2'-5')(A), synthesis (as determined at the optimal Mg<sup>2+</sup> concentration) is about 3.1-fold when the concentration of ATP is increased from 1 mM to 5 mM and about 3.5-fold when increased from 1 mM to 10 mM (Fig. 3B). The variation of the optimal Mg<sup>2+</sup> concentration with the concentration of ATP and the instability of the enzyme make it difficult to determine a precise K<sub>m</sub> for ATP.

The data in Fig. 3B seem to indicate that the concentration of ATP at which (2'-5')(A), synthesis is half-maximal is about 2 mM.

The optimal concentration of daRNA (i.e. poly(I)-poly(C) or reovirus daRNA in our experiments) increases with the enzyme concentration (Fig. 4A); in the presence of 0.4, 2, and 10 μg/ml of enzyme, the optimal poly(I)-poly(C) concentrations appear to be about 0.12, 1.2, and 7 μg/ml and the optimal reovirus daRNA concentrations about 0.23, 0.67, and 1.6 μg/ml. Thus, the optimal ratios of daRNA concentration/enzyme concentration (both in micrograms/ml) in the above cases are 0.30, 0.62, 0.70, 0.57, 0.48, and 0.16. Since the peaks of optimal daRNA concentrations are rather broad (see Fig. 4), only the following approximation can be proposed: (2'-5')(A), synthesis is close to optimal if the concentration of poly(I)-poly(C) or
to the enzyme concentration (Fig. 5); the shape of the curve of the rate versus enzyme concentration is sigmoidal. It will have to be established if this is a consequence of a cooperative interaction between enzyme subunits. Using as a basis for our calculation the amount of (2'-5')(A)ₙ synthesized by 2 μg/ml of enzyme from 10 μM ATP at 16 μM Mg²⁺ in 2 h (see Fig. 3A), we calculated the turnover number of (2'-5')(A)ₙ synthetase. The number obtained is 6.9 molecules of ATP converted to (2'-5')(A)ₙ enzyme/molecule/s. This is an underestimation of the real value because of the rate of the reaction decreases during the incubation in consequence of the inactivation of the enzyme (see Fig. 1).

(2'-5')(A)ₙ synthetase activated by dsRNA catalyzes the release of PP from ATP—(2'-5')(A)ₙ synthetase catalyzes the release of PP, from [γ-³²P]ATP. This release depends on the presence of both enzyme and dsRNA (Table I). The [γ-³²P]ATP used in this experiment was contaminated with small amounts of P₁. The amount of P₁ was not increased by (2'-

Reovirus dsRNA in the reaction mixture is half as much as that of the enzyme. The validity of this approximation was not tested, however, for cases in which the enzyme concentration was below 0.4 μg/ml or above 10 μg/ml. The stimulation of (2'-5')(A)ₙ synthetase activity decreases at dsRNA concentrations above the optimal (Fig. 4). This decrease cannot be overcome by increasing the concentration of Mg²⁺ (not shown).

Although E. coli 16 S ribosomal RNA (36) and mouse EAT cell tRNA contain double-stranded segments (37), if tested at 1, 3, 10, 30, or 100 μg/ml, they do not substitute for poly(I)-poly(C) in activating the enzyme. Moreover, at the above concentrations, these RNAs do not decrease the activity of the enzyme in the presence of 5 μg/ml of poly(I)-poly(C) (not shown).

The rate of (2'-5')(A)ₙ synthesis is not linearly proportional
5'-P(A)n synthetase in either the presence or absence of dsRNA. This indicates that the enzyme, even if activated, does not cleave PP, to P,

**Stoichiometry of (2'-5') (A)n Synthesis**—To determine the molar ratio between ATP cleaved, (2'-5') (A)n, synthesized, and PP, released, we performed the following sets of reactions (Table II): we incubated a mixture of [γ-32P]ATP and [α-32P]ATP (in a ratio of cpm/cpm = 1.08:1) with (2'-5') (A)n synthetase with (or without) dsRNA. This resulted in the formation of (2'-5') (A)n (α- and γ-labeled) and PP, (γ-labeled). Chromatographic fractionation and counting of the radioactivity in the fractions allowed the determination of the amount of radioactivity in the PP, released in response to the activation of the enzyme by dsRNA. This corresponded to 21% - 3.2% = 17.8% of the total radioactivity in the reaction mixture. To determine the amount of (2'-5') (A)n synthesized, we treated an incubated reaction mixture with alkaline phosphatase to remove the 5'-terminal triphosphate from ppApApA, and convert it to core (ApApA), α-labeled. The amount of radioactivity in the core was 16% of the total radioactivity in the reaction mixture. Based on these data, we calculated the molar proportion of PP, released to AMP moieties incorporated into the core moiety (2'-5') (A)n: 17.8%:16% = 1.1. Correcting this ratio by division with the factor 1.08 (the ratio of γ-32P to α-32P label) gives 1.1:1.08 = 1.03. This indicates that for every mole of AMP residues incorporated into (2'-5') (A)n core, 1 mol of PP, is released.

The Equilibrium of the Reaction Catalyzed by (2'-5') (A)n Synthetase Favors Synthesis—With enzyme at a low concentration (e.g. 0.5 μg/ml), only a small part of the ATP added was converted to (2'-5') (A)n, in our conditions (Fig. 1). This is apparently in consequence of the inactivation of the enzyme during incubation. About 90% of the ATP was converted to (2'-5') (A)n when the enzyme was used at an intermediate concentration (2 μg/ml) and the reaction mixture was supplemented with Triton X-100 (0.2%, v/v) to accelerate the reaction (Fig. 1). When tested at a high concentration (11 μg/ml), the enzyme can convert to (2'-5') (A)n, the large majority of ATP: 96.3% in one experiment and 98% in a second experiment (Table III).

As shown earlier (Table I), PP, is formed during the reaction and is not cleaved further by the enzyme. The accumulated PP, is, however, not inhibitory: even at a concentration as high as 7.5 mM it does not decrease the rate of conversion of 1 mM ATP to (2'-5') (A)n if tested in the presence of 16 mM Mg2+ (Fig. 6). If assayed at 8 mM Mg2+, even 2.5 mM PP, impairs the activity of the enzyme. This occurs most probably in consequence of the complexing of Mg2+ ions by PP.

We also tested for the reverse reaction of (2'-5') (A)n synthetase, i.e. the pyrophosphorolysis of (2'-5') (A)n. For this test, the complete reaction mixture included 0.33 mM ppApApA (unlabeled), 1 mM [32P]PP, 2 μg/ml of (2'-5') (A)n synthetase, 5 μg/ml of poly(I)-poly(C), 1 mM ATP, and 0.1 mM AMP. Incubation was at 30°C for 2, 3, and 17 h. Reaction mixtures were also incubated in the above conditions without one, two, or three of the following substances: poly(I)-poly(C), ATP, and AMP. The fraction of PP, (if any) incorporated into ATP was less than 0.1%, i.e. not significantly above the background level of our tests (see "Experimental Procedures").

All these results indicate that the equilibrium of the reaction catalyzed by (2'-5') (A)n synthetase is strongly in favor of synthesis.

**Effects of Incubation Conditions on the Size Distribution of (2'-5') (A)n**—As reported earlier, the chain length of (2'-5') (A)n produced by the purified enzyme extends from the dimer to about the pentadecamer. The size distribution

| Incubation | Reaction mixture A | Reaction mixture B | Reaction mixture C | Reaction mixture D |
|------------|-------------------|-------------------|-------------------|-------------------|
| Poly(I) | 12,400 | 5,600 | 87,100 |
| Poly(I) | 104,600 | 136,700 |
| Poly(I) | 33,100 (21%) | 5,000 (3.2%) | 1,700 |
| Poly(I) | 7,300 | 10,700 | 900 |
| Total | 157,400 | 154,400 | 106,700 |

"The amount of P, increased during the incubation in the complete reaction mixture in this experiment. We do not know the cause of this. No such increase occurred in several other experiments (see for example Table I and Table III).

The numbers in parentheses are per cents of the total counts per min in the form of PP, or core, respectively.
Table III

Equilibrium of (2'→5') (A)n synthesis

The standard reaction mixtures were supplemented with 17 mM Hepes/KOH (pH 7.5), 125 mM KCl, 8 mM Mg(OAc)2, 1 mM [α-32P]ATP, 11 μg/ml of (2'→5') (A)n synthetase, and, if so indicated, 5 μg/ml of poly(I)-poly(C) and/or 0.2% (v/v) Triton X-100. (Triton X-100 was found to accelerate the reaction approximately 2-fold.) Incubation was for 6 h. Thereafter, the reaction mixtures were heated at 95°C for 3 min to inactivate the enzyme, and were cooled and clarified by a 15-s centrifugation in the microfuge (Eppendorf). To convert (the unreacted) ATP to ADP, aliquots of the clarified solutions were supplemented with an equal volume of a second solution containing 0.1 pg/ml of hexokinase (EC 2.7.1.1., 125 mM Mg(OAc)2, and 10 mM Hepes/KOH (pH 7.5), 125 mM KCl, 5 mM Mg(OAc)2) and were incubated at 30°C for 30 min. This conversion of ATP was needed because, in the chromatographic analysis, the bulk of (2'→5') (A)n (i.e. dimers to pentamers) is separated from ADP but not from ATP. Aliquots from each reaction mixture were applied to a PEI plate which was developed with 0.75 M K phosphate (pH 3.5). P1, AMP, ADP, and ATP were used as region markers. The chromatogram was cut into regions which were eluted with 1 N HCl and counted. Substances eluted from the AMP and ADP regions (in track A) were further analyzed by homochromatography (31) to separate AMP and ADP from (2'→5') (A)n (some of which, less than 20%, also migrates in these regions; not shown). The homochromatogram revealed that 75% of the labeling in the "ADP + AMP" region is in (2'→5') (A)n, mostly in the hexamer to hexadecamer range and 25% in ADP and AMP. We also established that 4% of the total labeling in the [α-32P]ATP preparation was present as ADP and AMP already at the start of the incubation (not shown). The substance designated as (?) was present as an impurity in the [α-32P]ATP and was not identified. There was no discrete labeled spot in the region termed track background. Calculation of the per cent of ATP converted to (2'→5') (A)n in reaction mixture A, 12,801/18,205 = 84.1%. (This is the per cent of counts per min in ATP in the reaction mixture first with alkali and then with bacterial alkaline phosphatase (Fig. 7). The treatment with alkali degrades (2'→5')(A)n synthetase, and, if so indicated, 5 μM of (2'→5')(A)n, synthesized together with ATP, can be detected because the phosphodiester linkage connecting the 2' or 3'-OH of such a moiety to the adjacent adenylate (or deoxyadenylate) moiety is resistant to cleavage by alkali. (This is the case since alkaline hydrolysis of phosphodiester linkages in nucleic acids requires the participation of both 2' and 3'-hydroxyl group of the ribose moiety.) Thus, the treatment with alkali of an oligoadenylate containing a deoxyadenylate residue (although not in the 3'-terminal position) should generate oligonucleotides. For example, pppApAp would be produced if an oligoadenylate digested contained one deoxyadenylate moiety at its 5' terminus. (A stands for a 2' or 3'-deoxyadenosine moiety.) The data in Fig. 7 reveal that the pppApAp is produced upon the treatment with alkali of (2'→5')(A)n, synthesized in the presence of 3'-deoxyATP but not of that produced in the presence of 2'-deoxyATP. Moreover, as expected, the treatment of the pppApAp with bacterial alkaline phosphatase results in the conversion to A (Fig. 7, track E). These data indicate that in our conditions (2'→5')(A)n synthetase can link adenylic moieties to the 2'-hydroxyl of 3'-deoxyATP, but not to the 3'-hydroxyl of 2'-deoxyATP. 3'-DeoxyATP is, however, a poor competitor of ATP in initiating (2'→5')(A)n chains. The numbers of chains initiated by the two nucleotides are similar although the concentration of 3'-deoxyATP was 2500 times higher than that of ATP in the reaction mixture (Fig. 7, track B). The attachment of 3'-deoxyadenylate moieties (if any) into internal positions of oligoadenylates was below the level of detection in the test.

We tested in one experiment if the enzyme can use CTP, GTP or UTP as substrate. Using the standard reaction conditions (i.e. 2 μg/ml of enzyme, 1 mm ribonucleoside triphosphate), but a 10-n incubation, we found a slow oligomerization of UTP tested alone and a somewhat faster oligomerization of CTP when tested in the presence of 1 mM ATP. The
Synthesis of (2'-5')(A)n

In our studies on purified (2'-5')(A)n synthetase, we used poly(I)-poly(C) in solution rather than bound to paper (29, 31). We have chosen this although the enzyme is (at least if tested at low concentrations, i.e. about 11 μg/ml or lower) much less stable if activated by paper-bound poly(I)-poly(C) than if activated by paper-bound poly(I)-poly(C) (not shown). We have accepted the lesser stability because the initial velocity of the enzyme is about 22 times higher in the presence of free poly(I)-poly(C) than in that of paper-bound poly(I)-poly(C) (not shown).

As noted earlier, maximal or close to maximal activation of the enzyme in our conditions requires poly(I)-poly(C) or reovirus dsRNA at about half the concentration of the enzyme if expressed in terms of v/v. Taking the molecular weight of the enzyme to be 105,000 (Ref. 31; as determined by polyacrylamide gel electrophoresis in the presence of SDS) and the molecular weight of a base pair in poly(I)-poly(C) as 668, it can be calculated that this corresponds to about 79 base pairs in RNA/molecule of enzyme. This ratio is in line with the finding that poly(I)-poly(C) has to be at least 65 to 80 base pairs long to activate the enzyme maximally (30).

The specific activity of the enzyme decreases if the concentration of dsRNA is increased above the optimal value. It remains to be established whether or not this reflects the need for the binding of 2 or more enzyme molecules to adjacent sites on dsRNA for maximal activity, i.e. cooperativity. (The probability of the binding to adjacent sites on dsRNA of 2 or more enzyme molecules might be diminished if the dsRNA concentration is increased above the level allowing the attachment to dsRNA of all the enzyme molecules.) The sigmoidity of the plot of (2'-5')(A)n produced versus enzyme concentration (Fig. 5) is consistent with a cooperative behavior.

The data presented allow the formulation of the following equation for the formation of 1 molecule of (2'-5')(A)n

\[(n + 1) \text{ ATP} = (2'-5')\text{ppApa} + \text{ ATP}

The equilibrium of the reaction catalyzed by (2'-5')(A)n synthetase is shifted toward synthesis: about 96 to 98% (if not more) of the ATP can be converted to (2'-5')(A)n by the enzyme. This is remarkable since the enzyme does not seem to cleave further the PP, produced in the reaction. It is curious that the extent, if any, of the reverse reaction of (2'-5')(A)n synthesis, i.e. of the pyrophosphorylosis of (2'-5')(A)n, was below the level of detection in our conditions. Furthermore, we did not detect any exchange of [32P]PP into ATP when tested in the presence of (2'-5')(A)n, and/or ATP and/or AMP. Elucidation of the molecular basis of these characteristics of the enzyme will require further experiments.

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REFERENCES

1. Baron, S., and Dianzani, F., eds. (1977) Tex. Rep. Biol. Med. 35, 1-573

2. Stewart, W. E., II (1979) The Interferon System, Springer-Verlag, Vienna

3. Farrell, P. J., Broeze, R. J., and Lengyel, P. (1979) Nature 279, 523-525

4. Ball, L. A. (1979) Virology 94, 282-296

5. Knight, E. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1824-1827

6. Gupta, S. L., Rubin, B. Y., and Holmes, S. L. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4817-4821

7. De Ley, M., Billau, A., and De Sorom, P. (1979) Biochem. Biophys. Res. Commun. 89, 701-705

8. Farrell, P. J., Sen, G. C., Dubois, M. F., Ratner, L., Slattery, E., and Lengyel, P. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5893-5897

9. Slattery, E., Ghosh, N., Samanta, H., and Lengyel, P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4778-4782

10. Hovanessian, A. G., and Kerr, I. M. (1979) Eur. J. Biochem. 93, 515-526

11. Kimchi, A., Shulman, L., Schmidt, A., Chernajovsky, Y., Fradin, A., and Revel, M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3298-3302

12. Baglioni, C. (1979) Cell 17, 255-264

13. Lengyel, P., Desrosiers, R., Broeze, R., Slattery, E., Taira, H., Dougherty, J., Samanta, H., Pichon, J., Farrell, P., Ratner, L., and Sen, G. (1980) in Microbiology 1980 (Schlessinger, D.) pp. 219-226, American Society of Microbiology, Bethesda, Md.

14. Lewis, J. A., Falcoff, E., and Falcoff, R. (1978) Eur. J. Biochem. 86, 497-509

15. Samuel, C. E. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 600-604

16. Ball, L. A., and White, C. N. (1979) in Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators (Koch, G., and Richter, D.) pp. 303-317, Academic Press, New York

17. Ratner, L., Wiegand, R. C., Farrell, P. J., Sen, G. C., Cabrer, B., and Lengyel, P. (1978) Biochem. Biophys. Res. Commun. 81, 947-954

18. Clemens, M. J., and Williams, B. R. G. (1978) Cell 13, 565-572

19. Baglioni, C., Minks, M. A., and Maroney, P. A. (1978) Nature 273, 684-686

20. Kerr, I. M., and Brown, R. E. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 256-260

21. Williams, B. R. G., Golgher, R. R., Brown, R. E., Gilbert, C. S., and Kerr, I. (1979) Nature 282, 582-586

22. Williams, B. R. G., and Kerr, I. M. (1978) Nature 276, 88-90

23. Williams, B. R. G., Golgher, R. R., and Kerr, I. M. (1979) FEBS Lett. 105, 47-52

24. Hovanessian, A. G., Wood, J., Meurs, E., and Montagnier, L. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3261-3265

25. Kimchi, A., Shure, H., and Revel, M. (1979) Nature 282, 849-851

26. Hovanessian, A. G., Brown, R. E., and Kerr, I. M. (1977) Nature 267, 515-526
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268, 537-540
27. Minks, M. A., Benvin, S., Maroney, P. A., and Baglioni, C. (1979) *J. Biol. Chem.* 254, 5058-5064
28. Ball, L. A., and White, C. N. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 1167-1171
29. Stark, G. R., Dower, W. J., Schimke, R. T., Brown, R. E., and Kerr, I. M. (1979) *Nature* 278, 471-473
30. Minks, M. A., West, D. K., Benvin, S., and Baglioni, C. (1979) *J. Biol. Chem.* 254, 10180-10183
31. Dougherty, J. P., Samanta, H., Farrell, P. J., and Lengyel, P. (1980) *J. Biol. Chem.* 255, 3813-3816
32. Glynn, I. M., and Chappell, J. B. (1964) *Biochem. J.* 90, 147-149
33. Sen, G. C., Taira, H., and Lengyel, P. (1978) *J. Biol. Chem.* 253, 5915-5921
34. Ginsburg, D., and Steitz, J. A. (1975) *J. Biol. Chem.* 250, 5647-5654
35. Egg, A. (1978) Ph.D. dissertation, University of Zurich
36. Ehresmann, C., Stiegler, P., Mackie, G. A., Zimmermann, R. A., Ebel, J. P., and Fellner, P. (1975) *Nucleic Acids Res.* 2, 265-302
37. Singhal, R. P., and Fallis, P. A. M. (1979) *Prog. Nucleic Acid Res. Mol. Biol.* 23, 227-290