Structural requirements of polynucleotides for the activation of (2'-5')A_n polymerase and protein kinase

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ABSTRACT

Two enzymatic pathways are involved in the inhibitory effects of double-stranded (ds)RNA on protein synthesis in cell extracts derived from interferon-treated human fibroblasts or HeLa cells, an oligonucleotide polymerase that synthesizes (2'-5')A_n from ATP and a protein kinase that phosphorylates the α subunit of initiation factor eIF-2 as well as a polypeptide of M_r = 72,000. We have now evaluated the activation of both the (2'-5')A_n polymerase and protein kinase by a large variety of polynucleotides, including natural and synthetic dsRNAs, homopolymers, alternating copolymers, triple-stranded polymers, purine-purine duplexes and purine-pyrimidine duplexes with modifications at either the pyrimidine or ribose moieties. All these polynucleotides have been the subject of previous interferon induction studies. Some polynucleotides, i.e. (I)_n.(C)_n and mycophage dsRNA, which have been recognized as excellent interferon inducers, were also potent activators of both (2'-5')A_n polymerase and protein kinase, whereas non-inducers such as (A)_n.(X)_n and (A)_n.(br⁵U)_n did not activate either the kinase or the polymerase. However, some polymers like (I)_n.(br⁵C)_n, (dIfI)_n.(C)_n and (dIcI)_n.(C)_n, while potent interferon inducers and kinase activators, behaved poorly as activators of the (2'-5')A_n polymerase. Other polymers, i.e. (dAfl)_n.(U)_n and (A)_n.(U)_n. (I)_n, that do not induce interferon, activated the kinase but not the polymerase. Finally, (I)_n.(s²C)_n, a relatively potent interferon inducer, did not activate either kinase or polymerase. These findings indicate that there is no simple relationship between the interferon-inducing ability of dsRNAs and their stimulating effects on (2'-5')A_n polymerase and protein kinase activity.

INTRODUCTION

Double-stranded RNAs (dsRNAs) of either natural or synthetic origin are very effective interferon inducers (1) and also potent inhibitors of protein synthesis in cell-free systems prepared from rabbit reticulocytes (2) or interferon-treated cells (3). At least two dsRNA-dependent enzymatic activities may account for the inhibitory effects of dsRNAs on protein synthesis: (i) a protein kinase which phosphorylates and thereby inactivates the α subunit of initiation factor eIF-2, and (ii) an oligonucleotide polymerase, referred to as (2'-5')A_n polymerase, which synthesizes from ATP a series of (2'-5')A_n (pppA(2'p5'A)_n2'p5'A_{OH}) oligonucleotides, which may in turn activate a latent

endonuclease that degrades mRNA (4,5).

The hypothesis has been advanced that either of the two dsRNA-dependent enzymes, the protein kinase or the (2'-5')A_n polymerase, or both, may be part of the cellular recognition system for dsRNA, hence play a major role in the interferon induction process (6,7). To establish a possible connection between interferon induction, on the one hand, and activation of either protein kinase or (2'-5')A_n polymerase, on the other hand, one may determine the dsRNA structure-function relationships for both phenomena.

There are certain similarities between the structural requirements of dsRNAs for interferon induction and for inhibition of protein synthesis (8,9): both effects are critically dependent on the molecular size of the homopolymer components, a stable base-paired double-helical structure and the presence of free 2'-hydroxyl groups in both strands of the duplex. These structure-function studies have been extended to the protein kinase and (2'-5')A_n polymerase systems, and it was ascertained that in terms of molecular size (6) and presence of intact 2'-OH groups (7) the requirements of dsRNAs for activation of protein kinase and (2'-5')A_n polymerase would be similar to those required for interferon induction. Based on their studies with partially and fully 2'-O-methylated derivatives of (I)_n.(C)_n, Minks *et al.* (7) suggested a biphasic model for the activation of protein kinase and (2'-5')A_n polymerase, similar to that proposed for interferon induction (10). The interferon induction process may indeed occur as a biphasic event, involving first the binding of a large segment of dsRNA to the cellular receptor (11), followed by the triggering of interferon induction by a smaller region of dsRNA (10).

The possible relationship between the interferon inducing ability of nucleic acids and their activating effect on protein kinase and (2'-5')A_n polymerase has been further examined in the present report with the aid of a large variety of polynucleotides. These polynucleotides included both natural and synthetic dsRNAs, homopolymers, alternating copolymers, triple-stranded complexes, purine-purine duplexes and purine-pyrimidine duplexes with modifications at either the pyrimidine bases or ribose moieties. From our studies, it has become evident that if there is any relationship between interferon induction and the protein kinase and (2'-5')A_n polymerase systems, it must be a complex one.

MATERIALS

The origin of the polynucleotides was as follows: Penicillium chrysogenum dsRNA, extracted from purified mycophages (12), and provided by R.J. Douthart

(Lilly Research Laboratories, Indianapolis, Indiana); f₂dsRNA, extracted from *Escherichia coli* infected with f₂ phage (13), and provided by L. Borecky (Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia); (A)_n·(U)_n, constituted with homopolymers obtained from P-L Biochemicals (Milwaukee, Wisconsin); (A)_n·(rT)_n and (A)_n·(br⁵U)_n, as described previously (11); (dAfl)_n·(U)_n and (dAcl)_n·(U)_n, both constituted with (U)_n obtained from P-L Biochemicals and (dAfl)_n (14) or (dAcl)_n (15) provided by M. Ikehara (Osaka University, Osaka-fu, Japan); (I)_n·(C)_n, constituted with homopolymers purchased from P-L Biochemicals; (I)_n·(br⁵C)_n, as described previously (16); (I)_n·(s²C)_n (17), supplied by K. Reuss (E. Merck, Darmstadt, Germany); (dIf1)_n·(C)_n and (dIcl)_n·(C)_n, both constituted with (C)_n obtained from P-L Biochemicals and (dIf1)_n (18) or (dIcl)_n (19) provided by M. Ikehara; (A-U)_n·(A-U)_n purchased from Biogenics Research Corporation (Chagrin Falls, Ohio). The triplexes (A)_n·(U)_n·(I)_n and (A)_n·(U)_n·(X)_n were constituted according to directions described previously (20,21), as was (A)_n·(X)_n (22). The (I)_n samples 427 and 429 were synthesized by M.N. Thang (Institut de Biologie Physico-Chimique, Paris, France); the latter was found active in inducing interferon (23) whereas the former was not. The complexes (A)_n·(rT)_n, (A)_n·(br⁵U)_n and (I)_n·(br⁵C)_n were provided by P.F. Torrence (National Institutes of Health, Bethesda, Maryland).

METHODS

Assays for (2'-5')A_n polymerase. The assay for (2'-5')A_n synthesis with unfractionated cell extract has been described in detail (24). Cytoplasmic extracts were prepared from HeLa cells or human fibroblasts treated for 18 hr with 200 units/ml of human fibroblast interferon (3 x 10⁵ units/mg; Interferon Working Group, National Cancer Institute, National Institutes of Health, USA). The reaction mixtures contained 5 µl of cell extract, 9 or 25 mM Mg(OAc)₂, 0.12 M KOAc, 20 mM Hepes/KOH buffer, pH 7.4, 4 mM fructose 1,6-diphosphate, 1 mM dithiothreitol, 5 mM (0.2 µCi) [³H]ATP and 20 µg/ml of polynucleotide in a total volume of 25 µl. After 1 hr incubation at 30°C, the (2'-5')A_n synthesized was separated from ATP by chromatography on DEAE-cellulose (24). The yield of (2'-5')A_n is expressed in nmol of AMP equivalents converted into oligo(2'-5')adenylate per hour, per A₂₆₀ unit of cell extract (25). All dsRNAs were tested with both HeLa cell and human fibroblast extracts.

The assay for (2'-5')A_n synthesis has also been carried out with partially purified enzyme preparations free of (2'-5')A_n-degradative (2'-phosphodiesterase) activity (26,27). These preparations were obtained by pelleting ri-

bosomes from 10 ml of interferon-treated HeLa cell extracts by 90 min centrifugation at 200,000 x g. The pellet was resuspended in 1 ml of 0.5 M KCl, 1.5 mM Mg(OAc)₂, 0.5 mM dithiothreitol, 20 mM Hepes/KOH, pH 7.4, and centrifuged again in the same way. The supernatant was dialyzed overnight against 25 mM KCl, 5 mM Mg(OAc)₂, 1 mM dithiothreitol, 10 % glycerol and 20 mM Tris/HCl, pH 7.5. The precipitate formed during dialysis was removed by centrifugation and the supernatant was chromatographed through a DEAE-cellulose column (0.5 x 10 cm) equilibrated with the same buffer; 0.5 ml fractions were collected and assayed for (2'-5')A_n synthesis. To assess whether there was any (2'-5')A_n-degradative activity in the latter fractions, aliquots of each fraction were incubated with (³H)(2'-5')A_n, prepared as previously described (24). (³H)(2'-5')A_n was recovered by DEAE-cellulose chromatography (24). There was no loss of (³H)(2'-5')A_n. Hence, the fractions were free of 2'-phosphodiesterase activity. Finally, these fractions were assayed for (2'-5')A_n synthesis after a 18 hr-incubation period at 30°C, in the presence of 10 mM (0.4 μCi) (³H)ATP, 20 μg/ml of polynucleotide (unless indicated otherwise) and the other components described above (in a final volume of 25 μl).

Assay for protein kinase. The protein kinase activity was quantitated by measuring the phosphorylation of the 72,000 M_r polypeptide, exactly as previously described (28). The polynucleotide concentration was 1 μg/ml. The protein kinase assays were carried out with HeLa cell preparations and run in parallel with the (2'-5')A_n polymerase assays.

RESULTS

In the present study, we investigated the activation of (2'-5')A_n polymerase and protein kinase by a variety of natural and synthetic polynucleotides that have all been the subject of interferon induction studies in the past (11,16,20-23,29-31). Table 1 shows that the two natural dsRNAs were excellent activators of both polymerase and kinase. The synthetic homopolynucleotide duplexes (A)_n.(U)_n and (I)_n.(C)_n were also excellent activators as previously reported (6), whereas the only alternating copolymer tested, (A-U)_n.(A-U)_n, was less active. All double-stranded polynucleotides containing modified bases, i.e. (A)_n.(rT)_n, (I)_n.(br⁵C)_n and (I)_n.(s²C)_n, failed to activate the synthesis of (2'-5')A_n in our standard assay (although (A)_n.(rT)_n and (I)_n.(br⁵C)_n were active with the purified (2'-5')A_n polymerase). Triple-stranded complexes and purine-purine duplexes were also inactive, whereas a preparation of (I)_n that was previously shown to induce interferon (23) was marginally active in promoting the synthesis of (2'-5')A_n. The same results were

Table 1. Activation of (2'-5')A_n polymerase and protein kinase by different polynucleotides

Polynucleotide*	(2'-5')A _n synthesized			Protein kinase activity
	by unfractionated cell extract		by purified enzyme free of 2'-phosphodiesterase	
	9 mM Mg(OAc) ₂	25 mM Mg(OAc) ₂	30 mM Mg(OAc) ₂	
	<u>nmoles/hr</u>	<u>nmoles/hr</u>	<u>nmoles/18 hr</u>	<u>units</u>
<u>Natural dsRNAs</u>				
<u>P.chrysogenum</u> dsRNA	40.6	35.7	NT	100
F ₂ phage dsRNA	39.4	28.8	NT	100
<u>(A)_n·(U)_n analogs</u>				
(A) _n ·(U) _n	21.7	28.3	NT	100
(A) _n ·(rT) _n	0	0	1.2	69
(A) _n ·(br ⁵ U) _n	0	0	0	0
(dAfl) _n ·(U) _n	0	0	0	73
(dAcl) _n ·(U) _n	0	0	0	0
<u>(I)_n·(C)_n analogs</u>				
(I) _n ·(C) _n	42.0	38.6	52.7	100
(I) _n ·(br ⁵ C) _n	0	0	7.5	42
(I) _n ·(s ² C) _n	0	0	0	0
(dIf1) _n ·(C) _n	0	0	0.6	91
(dIcl) _n ·(C) _n	0	0	0.8	95
<u>Alternating copolymer</u>				
(A-U) _n ·(A-U) _n	4.4	6.2	NT	54
<u>Triple-stranded complexes</u>				
(A) _n ·(U) _n ·(I) _n	0	0	0	56
(A) _n ·(U) _n ·(X) _n	0	0	0	55
<u>Purine-purine duplex</u>				
(A) _n ·(X) _n	0	0	0	0
<u>Homopolymers</u>				
(I) _n 427	0	0	0	0
(I) _n 429	0.1	0.7	17.0	9.6

* In the (2'-5')A_n polymerase assays the polynucleotides were added at 20 µg/ml, except for (I)_n·(C)_n which was assayed at 0.1 µg/ml in the (2'-5')A_n polymerase reaction mixtures containing purified enzyme free of 2'-phosphodiesterase. In the protein kinase assays the polynucleotides were added at 1 µg/ml.

NT : not tested.

obtained with cell extracts prepared from interferon-treated HeLa cells and interferon-treated human fibroblasts.

When the polynucleotides were tested for protein kinase activation (Fig. 1), some interesting differences were observed. All the polymers activating the (2'-5')A_n polymerase could also activate the protein kinase, but some polymers inactive in the former assay were quite active in the latter (Table

Figure 1. - Activation of dsRNA-dependent protein kinase by various $(I)_n.(C)_n$ and $(A)_n.(U)_n$ analogs. Standard reactions were incubated as described in Methods. Phosphorylated proteins were fractionated by polyacrylamide gel electrophoresis and detected by autoradiography (28). The different lanes correspond to reactions containing : 1) no addition, 2) $(A)_n.(U)_n$; 3) $(A)_n.(rT)_n$; 4) $(I)_n$ (429); 5) $(I)_n.(C)_n$; 6) $(dIfI)_n.(C)_n$; 7) $(dIcI)_n.(C)_n$; 8) $(dAcl)_n.(U)_n$. The arrows indicate the positions of the 72,000 M_r polypeptide and of the eIF-2 α (38,000 M_r) polypeptide.

1). This was the case for $(A)_n.(rT)_n$, $(I)_n.(br^5C)_n$, and the 2'-modified polymers $(dIfI)_n.(C)_n$ and $(dIcI)_n.(C)_n$, all good interferon inducers (11,16,29,31). Other polynucleotides, such as $(dAfl)_n.(U)_n$ (31) and the two triple-stranded polymers (20,21) that do not induce interferon, activated the kinase but not the polymerase. Another polymer that can be considered as good inducer of interferon (17,30), $(I)_n.(s^2C)_n$, did not activate either the kinase or the polymerase. Other polymers, i.e. $(A)_n.(br^5U)_n$, $(A)_n.(X)_n$ and $(dAcl)_n.(U)_n$, that are inactive as interferon inducers (11,22,31), did not activate either enzymatic activity. Differences in phosphorylation were noted for both the 72,000 and 38,000 M_r polypeptides (Fig. 1). Only a faint band was visible in the 38,000 M_r region. A more pronounced band may have been obtained if eIF-2 were added to the reaction mixture (28).

The failure of some potent interferon inducers to activate the synthesis of $(2'-5')A_n$ was somewhat surprising in view of the close correlation between these two activities observed so far (6,7). Possibly, our standard assay for $(2'-5')A_n$ polymerase was not sensitive enough to detect a low level of enzy-

Figure 2. - Dependence on $(I)_n \cdot (C)_n$ concentration of $(2'-5')A_n$ synthesis with 2'-phosphodiesterase-free preparations of $(2'-5')A_n$ polymerase. The assays were carried out by incubating for 18 hr at 30°C 5 μ l of HeLa cell $(2'-5')A_n$ polymerase preparation (purified by DEAE-cellulose chromatography (26,27)) with 10 mM $[^3H]ATP$ (250 nmol, 288,150 cpm), 30 mM $Mg(OAc)_2$ and the other components described in Methods, in a final volume of 25 μ l. The amounts of $(2'-5')A_n$ synthesized are indicated on the y-axis; the amounts of $(I)_n \cdot (C)_n$ added to the assay mixtures are indicated on the x-axis.

matic activity. The standard assay for $(2'-5')A_n$ synthesis is carried out with crude cell extracts that contain a 2'-phosphodiesterase (26). This 2'-phosphodiesterase is inhibited to a large extent by the high concentration of ATP present in the assay for $(2'-5')A_n$ synthesis (32). However, no net accumulation of $(2'-5')A_n$ may be obtained with polymers that only marginally activate its synthesis. To evaluate this possibility, preparations of $(2'-5')A_n$ polymerase free of 2'-phosphodiesterase were incubated overnight with very low concentrations of $(I)_n \cdot (C)_n$ (0.01–0.03 g/ml). Linear synthesis of $(2'-5')A_n$ was noted with less than 1 ng of $(I)_n \cdot (C)_n$ per reaction mixture (25 μ l) (Fig. 2), whereas in our standard assay about 1 μ g/ml of dsRNA is required to observe net synthesis of $(2'-5')A_n$ (24). Several polymers, which were inactive in the standard assay, achieved a significant synthesis of $(2'-5')A_n$ when tested in the 2'-phosphodiesterase-free $(2'-5')A_n$ polymerase

assay (Table 1). This was the case for $(A)_n \cdot (rT)_n$, $(I)_n \cdot (br^5C)_n$, $(dIf1)_n \cdot (C)_n$, $(dIc1)_n \cdot (C)_n$ but not for $(I)_n \cdot (s^2C)_n$. Also the $(I)_n$ sample 429 that was marginally active in the standard assay, was quite active in the more sensitive assay. This polymer, however, was 1/880 as active as $(I)_n \cdot (C)_n$, as could be calculated from the dose response curve presented in Fig. 2. The other polymers were even less active; i.e. $(dIf1)_n \cdot (C)_n$ was only 1/20,000 as active as $(I)_n \cdot (C)_n$.

DISCUSSION

Previous investigations with mismatched and 2'-O-methylated $(I)_n \cdot (C)_n$ derivatives have indicated that the structural features of dsRNA relevant for the activation of the $(2'-5')A_n$ polymerase and protein kinase may be similar to those required for interferon induction (6,7). To further establish the relationship, if any, between interferon induction and activation of $(2'-5')A_n$ polymerase or protein kinase, a large variety of polynucleotides, which have all been the subject of extensive interferon induction studies in the past, were evaluated for their abilities to activate $(2'-5')A_n$ polymerase and/or protein kinase.

According to their activating effects on $(2'-5')A_n$ polymerase and protein kinase (Table 1) and their interferon-inducing properties (11,16,20-23,29-31), the polynucleotides could be grouped in five classes (Table 2). Class I would comprise those polynucleotides that are effective inducers of interferon, and, concomitantly, potent activators of either $(2'-5')A_n$ polymerase or protein kinase. Typical examples of this class of compounds are $(I)_n \cdot (C)_n$, $(A)_n \cdot (U)_n$ and the dsRNAs of natural origin (i.e. mycophage dsRNA and bacteriophage dsRNA).

The class II compound, $(I)_n \cdot (br^5C)_n$, $(A)_n \cdot (rT)_n$, $(dIc1)_n \cdot (C)_n$ and $(dIf1)_n \cdot (C)_n$ are also effective interferon inducers and potent activators of protein kinase, but differ from the class I compounds in that they are very poor activators of $(2'-5')A_n$ polymerase. In fact, $(I)_n \cdot (br^5C)_n$ and its congeners did not show any activation of the polymerase in our standard assay. They only showed weak activity after the $(2'-5')A_n$ polymerase had been purified from contaminating enzymes such as 2'-phosphodiesterase.

$(I)_n \cdot (s^2C)_n$ and another compound which was not part of the present study, $(dIz)_n \cdot (C)_n$, would belong to class III. Although $(I)_n \cdot (s^2C)_n$ and $(dIz)_n \cdot (C)_n$ are relatively potent interferon inducers (30,33), they do not stimulate either $(2'-5')A_n$ polymerase or protein kinase activity (Table 1 and ref. 34). The failure of $(I)_n \cdot (s^2C)_n$ to activate either the kinase or the polymerase

Table 2. Correlation between interferon induction, activation of (2'-5')A_n polymerase and activation of protein kinase by different polynucleotides

Polynucleotide	Activation of (2'-5')A _n polymerase*	Activation of protein kinase ⁺	Interferon induction [§]
<u>P.chrysogenum</u> dsRNA	+++	+++	+++
F ₂ phage dsRNA	+++	+++	+++
(I) _n .(C) _n	+++	+++	+++
(A) _n .(U) _n	+++	+++	+++
(A-U) _n .(A-U) _n	++	++	++
(I) _n 429	+	+	++
(I) _n .(br ⁵ C) _n	+	++	+++
(A) _n .(rT) _n	+	++	++
(dIc1) _n .(C) _n	+	+++	+++
(dIf1) _n .(C) _n	+	+++	+++
(I) _n .(s ² C) _n	-	-	++
(dAf1) _n .(U) _n	-	++	-
(A) _n .(U) _n .(X) _n	-	++	-
(A) _n .(U) _n .(I) _n	-	++	-
(dAc1) _n .(U) _n	-	-	-
(A) _n .(X) _n	-	-	-
(A) _n .(br ⁵ U) _n	-	-	-
(I) _n 427	-	-	-

* Activation of (2'-5')A_n polymerase was considered as +++, ++ or +, according to the amounts of (2'-5')A_n synthesized by unfractionated enzyme (Table 1, columns 1 and 2); +, if (2'-5')A_n was synthesized with the 2'-phosphodiesterase-free (2'-5')A_n polymerase only (Table 1, column 3), and -, if no (2'-5')A_n was synthesized with either unfractionated or purified enzyme (Table 1, columns 1-3).

+ Activation of protein kinase was considered as +++, ++, + or -, according to the units of protein kinase activity listed in Table 1, column 4.

§ Interferon induction was considered as +++, if maximum interferon titers were obtained in both highly sensitive induction systems (i.e. human diploid cells primed with interferon and superinduced with cycloheximide and actinomycin D) and relatively insensitive induction systems (i.e. mouse L cells primed with interferon); ++, if maximum interferon titers were obtained in the highly sensitive induction systems only; and -, if even the most sensitive induction systems did not yield appreciable interferon titers. For references to the interferon inducing activity of the polynucleotides see 11, 16, 20-23, 29-31.

may also explain why the compound was completely inactive as protein synthesis inhibitor in rabbit reticulocyte lysates (8) and extracts of interferon-treated mouse L cells (9).

Class IV would encompass the triple-stranded complexes $(A)_n \cdot (U)_n \cdot (X)_n$ and $(A)_n \cdot (U)_n \cdot (I)_n$ and the 2'-fluoro-substituted $(A)_n \cdot (U)_n$ derivatives $(dAfl)_n \cdot (U)_n$ (Table 2) and $(A)_n \cdot (dUfl)_n$ (34,35). $(A)_n \cdot (U)_n \cdot (X)_n$ and $(A)_n \cdot (U)_n \cdot (I)_n$ are inactive as interferon inducers (20,21) and so are $(dAfl)_n \cdot (U)_n$ (31) and $(A)_n \cdot (dUfl)_n$ (36). Yet, these compounds are quite effective activators of protein kinase, as shown here for $(A)_n \cdot (U)_n \cdot (X)_n$, $(A)_n \cdot (U)_n \cdot (I)_n$ and $(dAfl)_n \cdot (U)_n$ (Table 1) and elsewhere for $(A)_n \cdot (dUfl)_n$ (34,35). Neither $(A)_n \cdot (U)_n \cdot (X)_n$ nor $(A)_n \cdot (U)_n \cdot (I)_n$ nor $(dAfl)_n \cdot (U)_n$ activated the $(2'-5')A_n$ polymerase. This implies that the inhibitory effects of $(A)_n \cdot (U)_n \cdot (X)_n$ and $(A)_n \cdot (U)_n \cdot (I)_n$ on protein synthesis, as recorded in previous studies (9), could be fully explained by a stimulation of the protein kinase activity.

Finally, all polymers that are inactive as either polymerase or kinase activators or interferon inducers could be brought together in class V. Only a few examples of this class are listed in Table 2. Others examples are the homopolynucleotides $(A)_n$, $(U)_n$, $(C)_n$, the RNA-DNA hybrids $(I)_n \cdot (dC)_n$ and $(dI)_n \cdot (C)_n$ and the double-stranded DNA $(dI)_n \cdot (dC)_n$ (24).

Interestingly, no polynucleotide was found that activated the $(2'-5')A_n$ polymerase and failed to induce interferon or activate the protein kinase (Table 2). Thus, the activation of $(2'-5')A_n$ polymerase appeared to be the most delicate of the three biological parameters examined. It was more sensitive than any of the two other parameters to modifications of the dsRNA structure.

From an inspection of the data presented in Table 2 it is clear that there is no simple relationship between the interferon-inducing ability of polynucleotides and their stimulating effects on $(2'-5')A_n$ polymerase and protein kinase activity. This could perhaps be expected in view of the complexity of the $(2'-5')A_n$ polymerase and protein kinase systems. Indeed, both systems seem to be regulated by a number of factors. For instance, the net synthesis of $(2'-5')A_n$ is counteracted by a 2'-phosphodiesterase induced concomitantly with $(2'-5')A_n$ polymerase in the interferon-treated cell (37) and the effects of the protein kinase can be neutralized by a phosphoprotein dephosphatase (38). Because of these additional enzymatic activities, it may seem rather difficult to extrapolate from the structural requirements for the activation of isolated enzymes ($(2'-5')A_n$ polymerase, protein kinase) to the structural requirements for an intact cell response (interferon induction);

the more that the regulatory enzymes, akin the polymerase and kinase themselves, may be directly influenced (either inhibited or activated) by the polynucleotides.

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ABBREVIATIONS

dsRNA, double-stranded RNA; (2'-5')A_n, 2',5'-oligo(A), pppA(2'-5')A_n 2' p5'A_{OH}; (A)_n, poly(adenylic acid); (U)_n, poly(uridylic acid); (rT)_n, poly(ribothymidylic acid); (br⁵U)_n, poly(5-bromouridylic acid); (dUfl)_n, poly(2'-fluoro-2'-deoxyuridylic acid); (dAfl)_n, poly(2'-fluoro-2'-deoxyadenylic acid); (dAcl)_n, poly(2'-chloro-2'-deoxyadenylic acid); (I)_n, poly(inosinic acid); (C)_n, poly(cytidylic acid); (br⁵C)_n, poly(5-bromocytidylic acid); (s²C)_n, poly(2-thiocytidylic acid); (dIf1)_n, poly(2'-fluoro-2'-deoxyinosinic acid); (dIcl)_n, poly(2'-chloro-2'-deoxyinosinic acid); (dIz)_n, poly(2'-azido-2'-deoxyinosinic acid); (A-U)_n, (A-U)_n, an alternating copolymer of adenylic acid and uridylic acid; (X)_n, poly(xanthylic acid); (dI)_n, poly(2'-deoxyinosinic acid); (dC)_n, poly(2'-deoxycytidylic acid).

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