*TEMPLATE FUNCTIONS IN THE ENZYMIC FORMATION OF POLYRIBONUCLEOTIDES, III. APURINIC ACID AS TEMPLATE**

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In a previous communication[1] we have discussed some of the implications of the concept of a template and of its functions in the enzymic production of sequentially specific polynucleotides. It was shown that the highly polymerized DNA used, that of calf thymus, was extremely sensitive, in its efficiency as a template, to alterations of its physical structure. The composition of the polyribonucleotides formed by RNA polymerase appeared, however, to remain unchanged despite extensive losses of template activity as long as no chemical modifications of the template, such as the removal of purines from the DNA, took place. In the case of calf thymus DNA as the template, a very good agreement in composition between template and enzymic product was obtained. With the DNA from HeLa cells as the template this correspondence was less satisfactory.[2]

It is notoriously difficult to define perfection, whereas its absence is recognized more easily. We cannot even describe the ideal template as it may operate in the living cell; we can test the extremes of degradation. In the case of DNA these are probably the apurinic[3] and apyrimidinic[4] acids; structures that have not yet lost entirely the macromolecular character of the parent DNA, though their purine or pyrimidine constituents, respectively, have been eliminated completely. Previous studies from this laboratory have, in fact, demonstrated that the sequential arrangement of the pyrimidine nucleotides in apurinic acid[5] and that of the purine nucleotides in apyrimidinic acid [6, 7] do not deviate from the nucleotide sequence of the parent DNA.

The present communication gives a provisional account of the action of the apurinic acid of calf thymus DNA as a template for RNA polymerase and describes a few experiments with the apyrimidinic acid derived from the same DNA species.

Materials and Methods.—Three specimens of apurinic acid were used in the experiments, prepared from calf thymus DNA by the procedures described previously (ref. 3 and p. 341 of ref. 8). They had the expected composition, being free of purines and containing, as mole per cent per 100 gm atoms of DNA phosphorus: preparations 1 and 2, thymine, 28.5; cytosine, 21.5; preparation 3, thymine, 28.6; cytosine, 21.4. (The values for cytosine include 1.2–1.3% 5-methylcytosine.)

The specimen of apyrimidinic acid, kindly made available by Dr. H. Türler, was prepared from calf thymus DNA[6, 7] and contained: adenine, 28.1 per cent; guanine, 21.9 per cent.

The preparation of RNA polymerase from *E. coli* and the assay methods employed have been described in a previous communication.[1]

Results.—Template activity: Apurinic acid has been observed before[9] to act as a template in the enzymic formation of homopolymers; and also in our hands it proved effective in the synthesis of polyriboadenylic acid when ATP served as the only precursor. It was, however, also found to function as a template in the

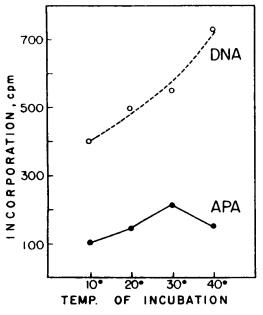

FIG. 1.—Effect of temperature of incubation on template activities of DNA and apurinic acid. The usual assay procedure was employed,[1] with 32 μg of enzyme protein and 200 mμmoles of each ribonucleoside triphosphate per assay tube. All four precursors were labeled with C^{14} (approx. specific activities, 135 cpm/mμmole). Quantities per assay tube of 100 μg of DNA and 200 μg of APA were used. Each point represents the average incorporation of two samples, incubated for 60 min at the specified temperatures.

presence of all four nucleoside triphosphates, though the composition of the products was not entirely reproducible in different experiments. This will be discussed below.

There may be several reasons for the greater variations observed in experiments with apurinic acid. One is its lability in solution. We have emphasized previously that the template activity of intact DNA diminishes very considerably upon its storage in solution.[1] Solutions of apurinic acid have been found to lose activity very rapidly. Another factor that distinguishes apurinic acid and DNA, and that may make for greater variability, when the former is used as the template, may be seen in the relation between activity and the temperature of incubation. As can be seen in Figure 1, the incorporation of radioactive precursors into the enzymic product insoluble in trichloroacetic acid increases with rising temperature when DNA is the template; with apurinic acid templates, however, there is an optimum temperature around 30°. This is reminiscent of observations on the behavior of short oligonucleotides as templates in the reaction with DNA polymerase.[10]

The effect of increasing concentrations of apurinic acid is shown in Figure 2. The level of template concentration at which incorporation reached a plateau varied

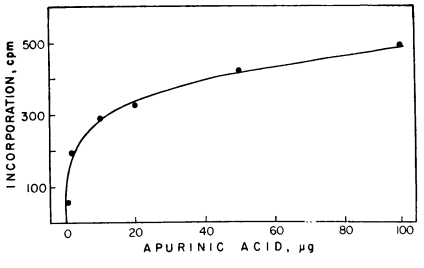

FIG. 2.—Dependence of template activity upon concentration of apurinic acid. The activity was tested with 25 μg of enzyme protein and 200 mμmoles of each ribonucleoside triphosphate per assay tube. All four precursors were labeled with C^{14} (approx. specific activities, 250 cpm/mμmole). Incubation at 30° for 30 min.

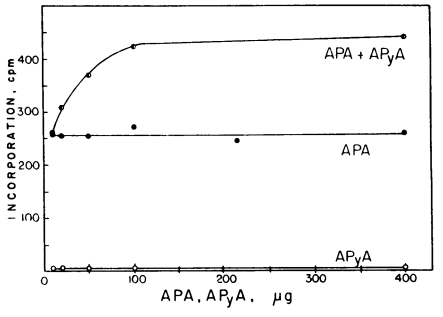

FIG. 3.—Synergistic effect of apurinic and apyrimidinic acids. The activity was tested with 21 μg of enzyme protein and 100 mμmoles of each ribonucleoside triphosphate, with ATP labeled with C^{14} (approx. specific activity, 1000 cpm/mμmole). The amounts of APA or APyA added as templates and, in the curve labeled APA + APyA, the amounts of each addition are indicated on the abscissa. Incubation at 30° for 60 min.

somewhat with the preparations of apurinic acid. The total activity of apurinic acid was usually in the range of 10–20 per cent of that of DNA under similar conditions of assay.

The available specimen of apyrimidinic acid induced no significant incorporation of radioactive precursors into polyribonucleotides when tested as the only template. It seemed, however, to have a synergistic effect on the template activity of apurinic acid (Fig. 3). At a concentration of apurinic acid at which the incorporation of nucleoside triphosphates apparently had reached a plateau under the conditions of the experiment, the simultaneous presence of apyrimidinic acid, in itself inactive, stimulated an incorporation, again concentration-dependent, up to a higher plateau. What remains to be ascertained is whether this effect denotes a specific interplay between the two extreme degradation products of DNA—a redintegration, as it were, of a more complete template—or whether we are dealing here merely with a stimulatory action comparable to that observed with polyamines.[11–13]

Composition of enzymic product: The composition of the material, insoluble in trichloroacetic acid, that is produced by RNA polymerase in the presence of apurinic acid, when all four nucleoside triphosphates are offered as precursors, is shown in Table 1. Three different preparations of apurinic acid, mentioned above, were used. Repeated experiments gave, qualitatively, the same results; but the quantitative agreement between different experiments, as can be seen in Table 1, is not very good. The incorporation of ATP[14] and GTP was to be expected, though the A/G ratio of the products is considerably higher than the T/C ratio of the template. CTP was, likewise predictably, not incorporated; but the significant uptake of UTP could not have been foreseen, since no adenine was detectable in the template preparations.

It must be kept in mind, especially when working with modified or degraded templates, such as apurinic acid, that the enzymic product available for examination represents the material insoluble in trichloroacetic acid and retained by the membrane filter. It is not unlikely that the products formed under the conditions discussed in this paper include smaller oligonucleotides that would escape detection with the assay methods used here. Techniques permitting a more complete characterization of the enzymic products are among the problems studied by us. One promising approach may be mentioned here.

Gel-filtration of template-product complexes: As can be seen in Figure 4, the peaks of UV absorption and of radioactivity are eluted together from a Sephadex column, when DNA is used as a template, though the molecular weight of the polyribonucleotide synthesized under these conditions has been found inferior to that of the DNA.[15] With apurinic acid as the template, a much broader peak of UV absorption is ob-

TABLE 1

APURINIC ACID AS TEMPLATE: COMPOSITION OF POLYRIBONUCLEOTIDE SYNTHESIZED BY RNA POLYMERASE

Base	Expt. 1 (%)	Expt. 2 (%)	Expt. 3 (%)
A	63	73	74
G	21	19	11
C	0	0	2
U	16	8	13

The assay methods have been described previously.[1] In each experiment, a different specimen of apurinic acid (preparations 1, 2, or 3) served as the template. The figures, based on the determination of the approximate specific activity of each precursor, refer to the proportions of each nucleotide in the enzymic product insoluble in trichloroacetic acid.

served, reflecting the polydispersity of the template; the peak of the radioactive enzymic product appears displaced toward the UV region of higher molecular weight. This may indicate that only apurinic acid molecules above a certain size can act as templates; but it could also be explained by the assumption that the poly- or oligonucleotides synthesized are able to form hybrids only with the larger fractions of apurinic acid.

Concluding Remarks.—The functions of a polynucleotide as an obligatory or auxiliary participant in a polymerase system may be those of an initiator or a primer or a template; or it may combine two of these functions or all three. We may try to define these terms which often are used rather loosely. (*a*) An *initiator* is required for the start of a polymerization process, without being incorporated covalently into the enzymic product; it has no directive influence on the composition of the product. An example may be seen in the initiation of the formation of polyriboadenylic acid by deoxypolyadenylic acid.[16] (*b*) A *primer* is a poly- or, more frequently, an oligonucleotide that starts, or facilitates, the polymerization process by providing a terminal free 3′-hydroxy group for the growing chain; it is incorporated into the product which in its composition does not have to reflect that of the primer. Though there are others, the best examples are found in systems involving the synthesis of polymers by polynucleotide phosphorylase.[17] (*c*) A *template* specifies the composition, and presumably the nucleotide sequence, of the product; it is an obligatory factor in the enzymic synthesis of sequentially specific polynucleotides. Examples have been cited in our previous communications.[1, 2]

While the function of intact DNA is at least definable, though much still remains obscure, how does the action of apurinic acid fit into this scheme? In promoting the

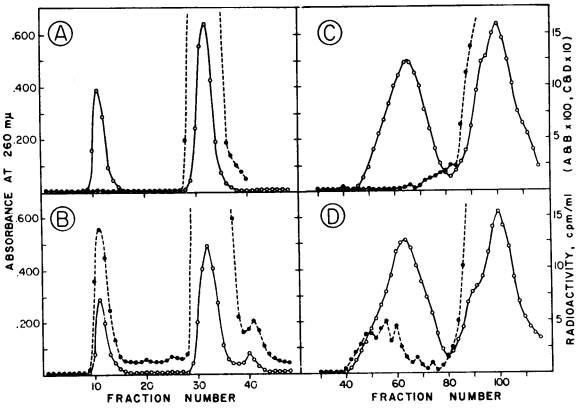

FIG. 4.—Separation of RNA polymerase products by gel-filtration. The incubation mixtures contained in a total volume of 5 ml the 10-fold amounts of all additions specified for the usual assay,[1] together with 250 μg of enzyme protein, 2 μmoles of each ribonucleoside triphosphate (only ATP labeled with C^{14}, approx. specific activity 1000 cpm/mμmole), and either 1 mg of calf thymus DNA [(*A*) and (*B*)] or 2 mg of APA [(*C*) and (*D*)]. In (*A*) and (*C*), enzyme was omitted. After incubation for 1 hr at 30° the mixture was applied to a column (50 × 4 cm) of Sephadex G-200. When APA was the template [(*C*) and (*D*)], the effluent was directly passed through a second column of the same size packed with Sephadex G-25. Elution was carried out with a Tris-HCl buffer (0.01 *M* Tris, 0.01 *M* $MgCl_2$, 0.0001 *M* EDTA, pH 7.0). In (*A*) and (*B*), 14-ml fractions were collected, and in (*C*) and (*D*) 10-ml fractions; samples of 1 ml served for the measurement of radioactivity.

enzymic synthesis of a homopolymer, polyriboadenylic acid, it acts presumably as an initiator; but this may not be its only role. Apurinic acid, as does the parent calf thymus DNA, contains a not inconsiderable quantity of tracts of polythymidylic acid: 3–4 per cent of the total thymine are present as pentathymidylic acid[18] and correspondingly smaller quantities as hexa, hepta, etc. These runs, when offered ATP as the only precursor, can undoubtedly act in a similar manner as the polythymidylic acid specimens that have been shown to promote the formation of polyriboadenylic acid.[16]

The action of apurinic acid in enzyme systems containing all four nucleoside triphosphates could be looked upon as that of a template, insofar as adenine and guanine are incorporated and cytosine is not. On the other hand, a simple transcription is excluded, since the A/G ratio of the product is much higher than the T/C ratio of the template; and, moreover, uracil is also incorporated into the product. It is possible that the products comprise both a faithful copy of the apurinic acid template, consisting of mixed A + G runs, and homopolymer chains of polyriboadenylic acid which latter act in turn as templates for the incorporation of UTP.[19] Experiments on these and related questions are in progress. They involve the elaboration of techniques for the additional characterization of the enzymic products, which are not limited to filtration or precipitation of the formed polynucleotides. In this respect, the gel-filtration technique mentioned before appears promising.

Summary.—When apurinic acid, instead of intact DNA, was used as template in the RNA polymerase system in the presence of all four ribonucleoside triphosphate precursors, a polyribonucleotide was synthesized containing a high proportion of adenine and smaller proportions of guanine and, unexpectedly, uracil. Cytosine was not incorporated. The rate of product formation depended on the concentration of apurinic acid, showed an optimum temperature of incubation at 30°, and was stimulated by the addition of apyrimidinic acid, which in itself had no template activity. Observations on the use of gel-filtration for the study of this product are mentioned briefly. These studies are being continued.

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[14] Abbreviations used: A, G, C, T, U designate adenine, guanine, cytosine, thymine, uracil; ATP, GTP, CTP, UTP, the corresponding ribonucleoside triphosphates. APA is apurinic acid. APyA apyrimidinic acid.

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