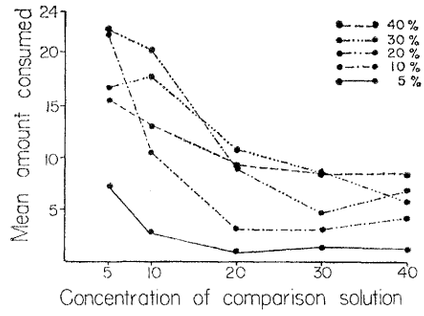

Fig. 2. Amount of each glucose solution consumed as a function of the concentration of the comparison solution.

decrease with increasing concentration was shown.

Position preferences were initially displayed by all subjects. However, the effect of position is considered negligible because the subjects consistently chose the solution with the higher concentration in the trials immediately preceding and following the trials in which identical solutions were presented. As days went by the position preferences decreased and in the case of two subjects actually reversed.

Of the models available for the scaling of paired comparison data, the obtained data best fit the assumption for Torgerson's condition B (*4*). Preference was determined by assuming that if an animal consumed more of one member of a pair of solutions, then the solution of which more was consumed was preferred. Table 1 shows the proportionate frequency for preference of each solution. The number of such preferences for the higher-concentration solution in each pair, expressed in terms of relative frequency, was the basic data to which the model was applied. The resulting scale is shown in Fig. 3, where the 40-percent solution has the highest scale value. If the model is applied to the data for amounts, the resulting scale again shows a similar order of preference. Both scales reflect the fact that more was consumed of the higher-concentration solution in each pair.

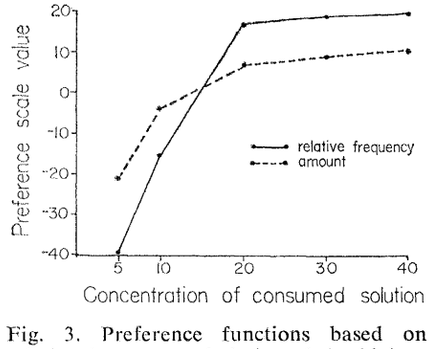

Fig. 3. Preference functions based on relative frequency of choice of the higher-concentration solution of a pair and on amount consumed of each member of a pair.

The goodness of fit of the model to the data was checked in Table 1 by finding the average deviation of proportions obtained from the raw data (*4*). In the case of the data for relative frequency, the average deviation was 0.098; in the case of the data for amounts consumed, it was 0.192. While these deviations are somewhat "large," the exploratory nature of this study must be borne in mind: that is, a relatively small number of observations was made on a small number of subjects.

The paired comparison method appears to yield a monotonically increasing preference function over the range of concentrations tested. Since the amounts of each solution consumed show a curve typical of such data, and since the scales produced by an appropriate paired comparison model show a curve which is qualitatively similar to operant data, it is suggested that the paired comparison method may provide a means of resolving differences in the results obtained with the two methods and may thus provide a useful tool in the determination of preferences in animals.

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Nucleic Acid Polymerases: Possible Subunit Structure

Abstract. *A hybrid polymerase which catalyzes the synthesis only of helical polynucleotides with one DNA-like strand and one RNA-like strand can be altered by certain treatments so that it will then synthesize DNA and RNA. There is evidence that the alteration involves a separation or rearrangement of polymerase subunits of several kinds. Two types of RNA polymerase activity have been found: one produces single chains, and the other, two complementary chains simultaneously. The latter type of RNA polymerase, the hybrid polymerase, and the DNA polymerase behave as though they were bifunctional, and each may be composed of two monofunctional subunits.*

Although a variety of nucleic acid polymerases have been observed in vitro, no attempt has been made to correlate the structures of the responsible enzymes. The striking similarities in the reactions catalyzed provide compelling evidence for structural similarities among the enzymes. All of the reactions require a polynucleotide template, divalent metal ions, and nucleoside-5′-triphosphate substrates; the products are polynucleotides which have been synthesized in a base-pairing fashion.

From studies on interconversions among the various polymerase activities presented here, we suggest that the nucleic acid polymerases are composed of subunits and that different activities may arise either by dissociation or rearrangement of the subunits. If this is true, then caution is required in interpreting the biological significance of results in vitro. The DNA-dependent synthesis of polyadenylate (*1*), for example, or the synthesis of other single homopolymers described here, may be due to enzymes which have lost or gained subunits on isolation. Furthermore, differences in primer requirements reported by others may also arise from such alterations.

Our work evolved from studies of a "hybrid" polymerase from *Escherichia coli*, which catalyzes the synthesis of hybrid molecules in vitro with DNA or helical polyribonucleotide templates (*2*). For example, with the homopolymeric complex poly(C+I) (*3*) as template and dGTP and CTP as substrates, the hybrid polymerase produces helical poly(dG+C), in which each polynucleotide chain is a homopolymer. Similarly, when the substrates are dCTP and GTP, the product is poly(dC+G). We have demonstrated that DNA polymerase from *E. coli*, in con-

trast, does not catalyze any reaction under these conditions, although it can utilize poly(C+I) as a primer when the appropriate substrates (both dGTP and dCTP) are present (*4*). The RNA polymerase reaction is also primed by poly(C+I) (*5*), but it can proceed in the presence of only one substrate (GTP or CTP). This has been shown by others with RNA polymerase and a poly(A+U) template (*6*). Thus, the use of the template poly(C+I) provides a ready basis for resolving various enzyme activities, since the particular substrate or combination of substrates present determines the type of activity manifested.

When the hybrid polymerase is freshly isolated, it can be obtained free from other polymerase activities (Table 1, column 1). Column 1 refers to the freshly purified preparation; all the treatments in columns 3 to 6 were carried out on the same preparation, but after it had been stored for several weeks at −20°C (column 2). In assays for polymerase activities, we employed poly(C+I) primer as the rate-limiting component. The reaction mixture was composed of 0.05*M* tris, *p*H 7.8; 0.005*M* $MgCl_2$; 0.005*M* mercaptoethanol; 50 mμmole poly (C+I), based on the phosphorus content; 50 mμmole of each substrate; and 0.1 ml of the hybrid polymerase solution (about 25 μg of protein), in a total volume of 1.0 ml. The total input of substrate labeled with C^{14} in position 8 of the base was 3 to 5 mμc. The mixture was incubated for 30 minutes at 37°C. The reaction was terminated, the product was precipitated with acid, and the radioactivity was determined as described (*2*).

Storage at −20°C for several weeks results in marked changes (Table 1, column 2): the hybrid activity decreases, whereas DNA and RNA polymerase activities increase; polyribonucleotides are synthesized in the presence of either one or both substrates, and there is also a small but real incorporation of dGTP alone. More GTP is incorporated in the presence than in the absence of CTP. Thus there may be two different polyribonucleotide-synthesizing activities, one producing one chain at a time (referred to here as RNA polymerase-1) and the other synthesizing two chains simultaneously (RNA polymerase-2) (*7*). The single-chain reaction itself seems to be inhibited by the presence of a second substrate (Table 1, columns 4 to 6, rows 3 and 5). The significantly greater extent of incorporation of the single substrate CTP, as compared to GTP, by the aged polymerase preparation (column 2) suggests that, unless these two activities are also different, the polymerase or the substrate, or both, binds more strongly to one chain of the poly(C+I) template than to the other.

Table 1. Interconversions of polymerase activities. The polymerase activities were derived by the various treatments from hybrid polymerase, which was prepared as described (*2*). The preparation was further purified by fractionation on a diethylaminoethyl cellulose column (20 cm by 12 cm^2). Each sample put on the column contained 180 mg of protein in 0.05*M* tris, *p*H 7.7, 0.01*M* $MgCl_2$, 0.01*M* mercaptoethanol, and 0.001*M* EDTA. The column was then washed with 50 ml of buffer (0.1*M* tris, *p*H 7.7, 0.01*M* mercaptoethanol, 0.01*M* $MgCl_2$), and eluted with 500 ml of 0.5*M* tris, *p*H 7.5, 0.01*M* mercaptoethanol, 0.01*M* $MgCl_2$. The flow rate was 30 ml/hr. Only the leading fraction of the first of four peaks was used in these experiments. However, almost identical transformations into the other polymerase activities were obtained from the hybrid polymerase preparation just prior to fractionation by chromatography.

Incorporation of the labeled substrate (mμmole) after the specified treatment *						Substrates	Type of polymerase activity
None	Aging	Urea†	Urea-Mg^{++}‡	EDTA§	EDTA-Zn^{++}‖		
0.72	9.5	0.72	0.66	0.53	2.4	dGTP–C^{14}, dCTP	DNA
34.0	7.2	0.72	.66	1.31	0.61	dGTP–C^{14}, CTP	Hybrid
0.36	9.8	1.5	.5	0.5	.3	GTP–C^{14}, CTP	RNA-1 and -2
.33	0.61	0.72	.64			dGTP–C^{14}	Polydeoxyribonucleotide
.31	5.2	4.5	.6	2.9	.8	GTP–C^{14}	RNA-1
.27	19.7	1.3	.6	1.3	.7	CTP–C^{14}	RNA-1

* Incorporation is in terms of radioactively-labeled nucleotide in an acid-precipitable polynucleotide. The background incorporation, in the absence of primer, corresponded to less that 0.02 mμmole. † The enzyme in 0.05*M* tris, *p*H 7.7, and 5×10^{-3}*M* mercaptoethanol was exposed to 6*M* urea overnight at 4°C. Urea was removed by exhaustive dialysis against the same solvent. The assays were performed after the enzyme solution had been free of urea for about 48 hours, at 4°C. ‡ The same as in the preceding set except that the dialysis solvent also contained 7×10^{-4}*M* $MgCl_2$. § The enzyme was dialyzed for 48 hours at 4°C against 0.05*M* tris, *p*H 7.7, 5×10^{-3}*M* mercaptoethanol and 1×10^{-3}*M* EDTA. A 1 : 20 dilution was used in the assay. ‖ The same as in the preceding set except that after exhaustive dialysis against EDTA the solution was then dialyzed for 24 hours at 4°C against 1×10^{-3}*M* Zn^{++}, 0.05*M* tris, *p*H 7.7, 5×10^{-3}*M* mercaptoethanol. A 1 : 20 dilution was used in the assay.

One possible interpretation of these results is that the hybrid polymerase is composed of DNA and RNA polymerase subunits which on storage dissociate and then reassociate in a different manner. If the two types of subunit both have association sites, it would not be surprising if interactions also occurred between like subunits. The synthesis of single chains such as polyguanylate from single substrates could then be attributed to single, dissociated subunits; on the other hand, the DNA polymerase, RNA polymerase-2, and the hybrid polymerase, each requiring two substrates with poly(C+I) as the primer, might arise from various aggregates of two subunits, which would be bifunctional. The subunits in question may or may not be further subdivided.

In order to gain some insight into the nature of the conversions to other activities upon storage at −20°C, a number of treatments known to disrupt protein structure were used. These include urea, ethylenediaminetetraacetate (EDTA), and freeze-thawing (Table 1). In general, inactivation occurs, and that brought about by EDTA can be partially reversed by divalent Zn, which produces an increase in DNA polymerase activity and a concomitant decrease in other activities.

Alternate freezing and thawing have been used to separate subunits of lactic dehydrogenase (*8*). A preparation of DNA polymerase containing a small proportion (about 10 percent) of RNA and hybrid polymerase activities was subjected to this treatment in 1*M* NaCl. There was a decrease in the DNA and RNA polymerase activities and an increase in the hybrid polymerase activity.

The activities which might be attributed to single subunits are decreased by conditions favoring aggregation but are relatively resistant to agents that may dissociate subunits (Table 1). This result is consistent with the hypothesis that nucleic acid polymerases are composed of dissociable subunits whose activities are a function of the state of aggregation.

On the other hand, the results could also be accounted for, albeit in a more complex fashion, if inhibitors are present. The decrease in hybrid polymerase activity and the concomitant increases in other polymerase activities after storage could be explained by the inactivation of a set of inhibitors which are unstable at −20°C. This would

mean that considerable amounts of both DNA and RNA polymerases with masked activities were in fact present in the hybrid polymerase preparation. But this seems unlikely since the three polymerase activities are obtained separately at different points in the preparation procedure. The presence of inhibitors in the purified preparation of hybrid polymerase, and their instability to storage and to the various treatments, would require rather unlikely properties for the inhibitory factors. Also, no inhibition is observed when DNA and RNA polymerase preparations are mixed. At present, therefore, we feel that the evidence points to a subunit structure.

Other observations can be explained readily by the subunit hypothesis. Since RNA polymerase is capable of copying only one chain of a DNA template, both in vivo (*9*) and in vitro (*10*), the native form would have just one active site. In contrast, DNA polymerase always synthesizes two chains simultaneously (*4*). One could, therefore, speculate that only monomeric RNA polymerase-1 and dimeric DNA polymerase are found inside the cell, the other polymerase activities being produced by dissociation and reassociation during or after isolation. The two subunits of the DNA polymerase would probably be oppositely oriented, like the polarities of the two template chains. Both DNA and RNA polymerase interact with the same template, but different sites of interaction have been suggested (*11*) by the differential action of actinomycin. The different sites may be related to the mono- or bifunctional character of the enzymes. Thus, one groove of the DNA helix may accommodate the dimeric DNA polymerase, which interacts with both chains, whereas monomeric RNA polymerase lies in the other groove.

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DNA Synthesis in Aluminum-Treated Roots of Barley

Abstract. *When cell division in barley roots is halted by treatment with aluminum, DNA synthesis continues. However, the type of DNA synthesized has an unusual base composition and is metabolically labile. A part of this labile DNA was found in the form of a hybrid composed of genetic DNA and labile DNA.*

The damaging effect of aluminum in the cationic form on root growth of many plant species (*1*) may be partly explained by an inhibition of cell division (*2*). The roots of barley are particularly sensitive to aluminum (*3*). We have treated the roots of barley, *Hordeum vulgare* var. Proctor, with aluminum sulfate ($10^{-3}M$) in water culture at *p*H 4.5. Examination of "squash" preparations of root apices stained with acetocarmine revealed that during this treatment there was a steady decline in the number of cells entering prophase, and after 24 hours mitotic figures were no longer seen (Fig. 1). The rate of cell division was not markedly affected in roots raised on a similar medium at *p*H 4.5 when no aluminum was added. The effects on the root growth of many species at this *p*H are only slight (*4*).

In the roots of wheat there are two physiologically distinct fractions of double-stranded DNA (*5*). One has a low molecular weight (2 to 3 $\times$ 10^5), and a mole percentage of guanine plus cytosine equal to 51; it accounts for some 20 percent of the total DNA in root and is metabolically labile. The other form, which we consider to be genetic DNA, has a high molecular weight (4 to 6 $\times$ 10^6) and a mole percentage of guanine plus cytosine equal to 41. This form of DNA is metabolically stable. Two such fractions have been characterized from the roots of *Zea mays* with orthophosphate-^{32}P and thymidine-^{14}C as DNA precursors (*6*).

The two forms of DNA can be separated by chromatography on a column containing methylated-albumin-kieselguhr (MAK) (*7*). The metabolically labile, low-molecular-weight DNA of wheat root can be eluted step by step with NaCl (0.5*M*) in phosphate buffer (*p*H 6.8, 0.05*M*). After these steps the metabolically stable high-molecular-weight DNA of wheat root can be eluted with NaCl (1.0*M*) in the same phosphate buffer.

We have studied the effect of aluminum treatment on the synthesis of these two DNA fractions with orthophosphate-^{32}P as a DNA precursor. Four batches of barley plants were grown on plastic mesh frames in water culture at *p*H 4.5, the medium containing the following in millimolar concentrations: KCl, 0.5; CaCl, 2.5; NaH_2PO_4, 0.05; $MgSO_4$,0.25; NH_4NO_3, 1.0; ferric citrate, 0.04; trace elements (*8*). When the roots had grown to approximately 10 cm in length, two batches of plants were transferred to a similar medium without phosphate and containing aluminum sulfate ($10^{-3}M$). The other two batches were transferred to this phosphate-free medium without the addition of aluminum sulfate.

After 48 hours there were no mitotic stages in the roots of the aluminum-treated plants although active division was taking place in the roots of the untreated control plants. After washing the roots, the four batches of plants were labeled for 4 hours with carrier-free $^{32}P_i$ (inorganic P). The roots from one control and one aluminum-treated batch of plants were then harvested. The two remaining batches of plants were given a "chase" treatment (48 hours) with sodium dihydrogen phosphate ($10^{-4}M$). Aluminum sulfate ($10^{-3}M$) was added to that batch of plants which had been aluminum-treated prior to the addition of isotope. The roots were then harvested, and DNA was extracted (*5*) and fractionated on a MAK column as described.

In the untreated roots of barley there are two DNA fractions corresponding to those found in wheat roots (*5*)