The Role of *Halobacterium cutirubrum* Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase Subunits in Initiation and Polymerization

By B. GREGORY LOUIS and P. S. FITT

Department of Biochemistry, University of Ottawa, Ottawa, Ont. K1N 6N5, Canada

(Received 7 October 1971)

1. The two subunits α and β of *Halobacterium cutirubrum* DNA-dependent RNA polymerase are required in equimolar amounts for RNA synthesis to occur *in vitro* at the maximum rate. 2. In the absence of bivalent cations no interaction occurs between α and β subunits or between the subunits and DNA. 3. Mn^{2+} causes the subunits to form a 1:1 complex that still does not bind to the template. 4. Mg^{2+} permits binding of the Mn^{2+} -mediated complex to DNA. 5. The complete enzyme, $\alpha\beta$, is inhibited by rifampicin and only the β subunit relieves the inhibition when added in excess. 6. Rifampicin-insensitive, template-dependent RNA synthesis occurs in the presence of protein α alone provided an oligonucleotide with a 5'-purine terminus is supplied as primer. 7. In the primed reaction with the α protein and an oligonucleotide, the template specificity is independent of the ionic strength, in contrast with the marked effect of salt concentration on the template specificity of the complete enzyme. 8. It is concluded that the β protein controls the specificity of chain initiation and the template specificity of the complete enzyme and also carries the rifampicin-binding site, whereas the catalytic site is on the α subunit.

In the preceding paper (Louis & Fitt, 1972) we described the purification to electrophoretic homogeneity and the further characterization of the DNA-dependent RNA polymerase [nucleoside triphosphate-RNA nucleotidyl transferase (DNA-dependent) EC 2.7.7.6] from the extreme halophile *Halobacterium cutirubrum*. Earlier studies (Fitt & Louis, 1970; Louis & Fitt, 1971a,b; Louis *et al.*, 1971) had shown that the enzyme was of unusually low molecular weight and we found (Louis & Fitt, 1971b) that it consists of two subunits, designated α and β , required in equimolar amounts for optimum activity. The subunits were both of similar, low molecular weight (about 18000) and did not associate in the 2.5M-KCl-1M-NaCl-10mM-tris-HCl buffer, pH 8.6, used in the physical studies and for storage. Further, the template specificity of the complete enzyme ($\alpha\beta$) was markedly affected by the ionic strength of the assay medium (Louis & Fitt, 1971b, 1972).

We present the results of studies of the roles of the two proteins in RNA synthesis and of the nature of their functional association. A preliminary report of some of these results has been published (Louis & Fitt, 1971c).

Experimental

Materials

Rifampicin was purchased from Mann Research Laboratories, New York, N.Y., U.S.A.; dinucleoside phosphates and oligonucleotides from Miles Labora-

tories, Inc., Kankakee, Ill., U.S.A. Streptolydigin was a gift of Upjohn Co., Kalamazoo, Mich., U.S.A. The sources of other materials are given in the preceding paper (Louis & Fitt, 1972).

Buffers. The pH of buffers refers to the value at 25°C. High-salt buffer has the composition: 2.5M-KCl-1M-NaCl-10mM-tris-HCl, pH 8.6.

Methods

Purification of *H. cutirubrum* DNA-dependent RNA polymerase. Pure proteins α and β were obtained as described by Louis & Fitt (1971a,b, 1972).

Enzyme assay. *H. cutirubrum* DNA-dependent RNA polymerase activity was determined by the method of Louis & Fitt (1972). The assay medium (0.1 ml) contained: glycine-NaOH buffer, pH 9.5, 10 μ M; native *H. cutirubrum* DNA, 30 μ g; [14 C]-ATP, 5 nmol; CTP, GTP and UTP, 15 nmol each; $MgCl_2$, 10 μ M; $MnCl_2$, 1 μ M; KCl, 150 μ M; NaCl, 60 μ M; enzyme, up to 3 units. Incubation was at 37°C for 1 h. Samples (up to 85 μ l) were streaked on Whatman no. 3 filter-paper strips (1.5 cm $\times$ 3 cm). The latter were placed in 7% (w/v) $HClO_4$ (10 ml/strip) and the suspension was swirled for 7 min on a rotary shaker. After aspiration of the liquid, the washing was repeated successively with 7% (w/v) $HClO_4$, 1% (w/v) $HClO_4$ and ethanol. The strips were dried in air and their radioactivities determined in a Beckman LS-230 liquid-scintillation counter with 0.8% (w/v) 2,5-diphenyloxazole in toluene (10 ml/strip) as scintillator. All results were corrected for quenching as described by Louis & Fitt (1971a) and

for incorporation into suitable controls. One unit was defined as the amount of enzyme catalysing incorporation of 1nmol of ATP into acid-insoluble material/h in these conditions.

Protein assay. Protein was determined spectrophotometrically by the method of Warburg & Christian (1942).

Analytical gel-filtration chromatography. A column (1.5cm×27cm) of Bio-Gel P-60 in high-salt buffer was packed as described by Louis *et al.* (1971), but without reversing the flow of buffer through the bed. Samples (1ml) were applied directly to the upper surface and rinsed on to the column with 1 ml of the appropriate buffer, care being taken not to exceed a hydrostatic pressure of 15cm. Eluant buffer changes were kept to a minimum and experiments involving Mn^{2+} were performed last. After each change of eluant, 30ml of the new buffer was run through the column before the next sample was loaded. Eluant buffer and sample compositions are shown in Tables 1 and 2. Fractions (1ml), were collected and their protein and nucleic acid contents determined from their E_{260} and E_{280} values. The average partition coefficient ($K_{av.}$) was calculated for each peak as previously described (Louis *et al.*, 1971; Fischer, 1969).

Results

*Influence of Mg^{2+} and Mn^{2+} on *H. cutirubrum* RNA polymerase subunits and on their interaction with DNA*

Our earlier studies (Louis & Fitt, 1971a) showed that both Mg^{2+} and Mn^{2+} (at optimum concentrations of 100 and 10mM, respectively) are required by *H. cutirubrum* DNA-dependent RNA polymerase. No reaction took place in the conditions of the standard assay if either cation was omitted. Physical studies of the two subunits, proteins α and β , and of the complete enzyme ($\alpha\beta$) had also demonstrated (Louis *et al.*, 1971; Louis & Fitt, 1971b) that there was no interaction between proteins α and β in high-salt buffer (2.5M-KCl–1M-NaCl–10mM-tris–HCl buffer, pH8.6), although they were required in equimolar amounts for optimum activity in the standard assay. Clearly, some interaction between the subunits and the template must occur in the reaction, so a study was made of the influence of Mg^{2+} and Mn^{2+} on the behaviour of the α and β subunits during polyacrylamide-gel filtration in high-salt buffer in the absence and presence of native *H. cutirubrum* DNA.

Table 1 shows the effect of Mg^{2+} and Mn^{2+} on the elution by high-salt buffer from a Bio-Gel P-60 column of samples containing the α and/or β subunit in the absence of DNA. In these conditions, a $K_{av.}$ of 0.62 represents a mol. wt. of about 18000 (Louis *et al.*, 1971). The two proteins did not associate either in high-salt buffer alone or in the presence of 100mM- $MgCl_2$. However, in the presence of 10mM- $MnCl_2$,

Table 1. Association of α and β in presence of Mn^{2+}

Samples containing protein α and/or protein β (80 μ g) were chromatographed on Bio-Gel P-60 and eluted with high-salt buffer with or without 10mM- $MnCl_2$ and/or 100mM- $MgCl_2$, as described in the Experimental section.

Sample	Bivalent cations	Protein peak	
		$K_{av.}$	Recovery ($\pm 5\%$)
α	—	0.62	100%
β	—	0.62	100%
$\alpha + \beta$	—	0.61	90%
$\alpha + \beta$	Mg^{2+}	0.62	90%
$\alpha + \beta$	Mn^{2+}	0.53	100%
$\alpha + \beta$	$Mn^{2+} + Mg^{2+}$	0.53	100%

all the protein emerged at a $K_{av.}$ of 0.53, corresponding to mol. wt. 36000. As the α and β subunits do not dimerize in the presence of Mn^{2+} (see below), this result indicates that a 1:1 complex of α and β subunits was formed in these conditions.

The effect of the metal ions on the binding of α or β subunits singly to native *H. cutirubrum* DNA was then examined. When DNA and either α or β protein were chromatographed together in the presence or absence of 100mM- $MgCl_2$ and/or 10mM- $MnCl_2$, the DNA was eluted at the void volume free from protein contamination, whereas the subunit concerned appeared at a position corresponding to its monomer molecular weight. Thus neither subunit can bind to DNA in the absence of the other or dimerize in the presence of Mn^{2+} (or Mg^{2+}).

Finally, the binding of the $\alpha\beta$ complex to DNA was studied (Table 2) by chromatography of samples containing the nucleic acid and both subunits. In the absence of the bivalent cations, or with 100mM- $MgCl_2$ alone, the DNA emerged at the void volume and the proteins at $K_{av.}$ 0.62, corresponding to the monomer molecular weight. In the presence of 10mM- $MnCl_2$ alone, the $\alpha\beta$ complex ($K_{av.}$ 0.53) formed, but it still did not interact with the DNA, which emerged at the void volume free from protein. However, when both 100mM- $MgCl_2$ and 10mM- $MnCl_2$ were present, the entire sample was eluted at the void volume. This result clearly indicates that in the presence of Mg^{2+} , the Mn^{2+} -mediated $\alpha\beta$ complex was bound to DNA, since omission of the latter (Table 1) led to the formation of the $\alpha\beta$ complex only and not of a large protein aggregate.

Inhibition of chain initiation by rifampicin

The antibiotic rifampicin is known to inhibit the formation of new RNA chains by *E. coli* RNA poly-

Table 2. *Binding of the $\alpha\beta$ complex to DNA*

The conditions of the experiment are described in the Experimental section and Table 1.

Sample	Bivalent cations	Nucleic acid peak		Protein peak	
		$K_{av.}$	Recovery ($\pm 5\%$)	$K_{av.}$	Recovery ($\pm 5\%$)
$\alpha + \beta + \text{DNA}$	—	0.00	100%	0.62	90%
$\alpha + \beta + \text{DNA}$	Mg^{2+}	0.00	95%	0.62	100%
$\alpha + \beta + \text{DNA}$	Mn^{2+}	0.00	100%	0.53	100%
$\alpha + \beta + \text{DNA}$	$\text{Mn}^{2+} + \text{Mg}^{2+}$	0.00	*	0.00	*

* Approx. 95% recovery of u.v.-absorbing material.

Table 3. *Inhibition of H. cutirubrum DNA-dependent RNA polymerase by rifampicin*

Standard assay mixtures (0.1 ml; see the Experimental section) containing unlabelled ATP (10 nmol), GTP (10 nmol), CTP (15 nmol), UTP (15 nmol) and 160 ng of each subunit were incubated for 10 min at 37°C. To each of the control assays was added 5 μl of a solution containing [^{14}C]ATP, [$\gamma\text{-}^{32}\text{P}$]ATP and [$\gamma\text{-}^{32}\text{P}$]GTP of high specific radioactivity. The other assays received a similar solution containing, in addition, 1.16 nmol of rifampicin. Incubation was continued for 20 min and the reaction was then processed by the standard procedure (Louis & Fitt, 1972).

Experiment	^{14}C incorporated (nmol) (elongation)	^{32}P incorporated (nmol) (initiation)	Ratio $^{14}\text{C}/^{32}\text{P}$
Control	0.68	0.057	12.0
+Rifampicin	0.19	0.00041	473

merase, but not the elongation of preformed chains (Lill *et al.*, 1970). As shown in Table 3, it has a similar effect on the DNA-dependent RNA polymerase of *H. cutirubrum*. Complete standard assay mixtures, containing unlabelled substrates and equimolar amounts of α and β , were preincubated to permit the initiation of new RNA chains to occur. Rifampicin was then added to the test solution together with [^{14}C]ATP, [$\gamma\text{-}^{32}\text{P}$]ATP and [$\gamma\text{-}^{32}\text{P}$]GTP. The control received the radioactive substrates, but no antibiotic. Although a significant, if decreased, rate of chain elongation was observed in the presence of rifampicin, very little $\gamma\text{-}^{32}\text{P}$ was incorporated and the chain elongation/chain initiation ($^{14}\text{C}/^{32}\text{P}$) ratio was 40 times that measured in the absence of the antibiotic.

Table 4 shows that addition of rifampicin at the start of the reaction inhibits the latter completely and that the effect of the antibiotic is not overcome by a large excess of α protein. However, the inhibition is relieved completely by excess of β protein, so that the latter must contain the rifampicin binding site and be involved in the initiation process.

The inability of either subunit to initiate RNA chains in the absence of the other was then demonstrated (Table 5). Standard assay mixtures were preincubated for 30 min with either α or β protein before

the addition of rifampicin and the other subunit. No elongation was observed in either case, indicating that no complex capable of acting as a nucleus for chain extension had been formed, although the expected high rate of incorporation was measured in the control assay that contained both subunits from the start of the experiment. Thus, neither subunit is an initiation enzyme capable of forming the first phosphodiester bond and supplying by itself dinucleotides as primers for chain elongation.

The effect of streptolydigin on the activity of the complete enzyme ($\alpha\beta$) was also examined. With 0.2 mM streptolydigin, double the concentration that inhibited the *Escherichia coli* RNA polymerase completely (Cassani *et al.*, 1970), there was no effect on the activity of the *H. cutirubrum* RNA polymerase. Thus, the latter resembles the *E. coli* enzyme in its sensitivity to rifampicin, but is like the $\text{T}_3\text{-}$ and $\text{T}_7\text{-}$ induced polymerases (Dunn *et al.*, 1971) in being unaffected by streptolydigin.

Primer-dependent RNA synthesis catalysed by protein α alone

No activity was observed in the standard conditions unless both α and β proteins were present (Louis &

Table 4. *Effect of excess of α or β on rifampicin inhibition*

Chain elongation (^{14}C incorporation) and initiation (^{32}P incorporation) were measured in the conditions of the standard assay with or without rifampicin. The incubation mixture (0.1 ml) contained: glycine-NaOH buffer, pH 9.5, 10 μmol ; native *H. cutirubrum* DNA, 30 μg ; [^{14}C]- and [γ - ^{32}P]-ATP, 10 nmol (5000 and 100000 d.p.m./nmol of ^{14}C and ^{32}P , respectively); [γ - ^{32}P]GTP, 10 nmol (100000 d.p.m./nmol); CTP and UTP, 15 nmol each; MgCl_2 , 10 μmol ; MnCl_2 , 1 μmol ; KCl , 150 μmol ; NaCl , 60 μmol ; proteins α and β , as indicated; rifampicin (0.20 nmol) where appropriate. For either subunit, 8000 ng represents 0.44 nmol. Incubation was at 37°C for 1 h, and the samples were prepared for the radioactivity assay as usual (see the Experimental section).

Protein (ng)		Rifampicin	^{14}C ATP incorporated (nmol)	^{32}P NTP incorporated (nmol)	Ratio $^{14}\text{C}/^{32}\text{P}$
α	β				
40	40	—	1.01	0.061	16.7
40	40	+	0	0	—
8000	40	—	1.00	0.060	16.7
8000	40	+	0	0	—
40	8000	—	1.00	0.057	17.8
40	8000	+	0.94	0.061	15.4

Table 5. *Inability of α and β singly to initiate RNA chains*

Standard assay mixtures containing protein α (160 ng) and/or protein β (160 ng) were incubated for 30 min at 37°C. The indicated additions were then made and incubation was continued for an additional 30 min. Samples were processed by the standard procedure to determine the incorporation of [^{14}C]ATP into an acid-insoluble form during the total incubation period.

Subunit in standard assay	Addition after 30 min	^{14}C ATP incorporation (nmol)
α	β + 1.16 nmol of rifampicin	0
β	α + 1.16 nmol of rifampicin	0
$\alpha + \beta$	1.16 nmol of rifampicin	2.8

Fitt, 1971b; and Table 5). However, if one of the dinucleoside phosphates ApA, ApU or GpU was included in a standard assay mixture containing subunit α alone (Table 6), synthesis occurred at a rate similar to that attained in the standard assay in the presence of both α and β subunits. The dinucleoside phosphate apparently acted as a primer (chain initiator) by supplying the first phosphodiester bond, because no initiation of new chains could be detected in these conditions. Further, in preliminary experiments in which crude [^3H]ApA was used as primer, ^3H as well as ^{14}C was incorporated into the product. The primed reaction was dependent on the presence of a DNA template, but unlike the unprimed reaction catalysed by the complete enzyme it was unaffected by rifampicin. Nearest-neighbour analysis of the product formed during primer-dependent synthesis catalysed by protein α on a poly[d(A-T)] template showed that the latter was copied accurately (Table 7).

No primer-dependent polymerization occurred in the presence of protein β alone and the dinucleoside phosphates CpG, CpC and CpU were not primers,

in agreement with the observation (Louis & Fitt, 1972) that chain initiation by the complete *H. cutirubrum* DNA-dependent RNA polymerase involves purine nucleoside triphosphates only.

The effect of variation in the length of an oligonucleotide primer was also investigated (Table 6) with the series $(\text{Ap})_n\text{A}$, $n = 1-5$, and either the very regular alternating copolymer poly[d(A-T)] or *H. cutirubrum* DNA as template. The length of the primer did not alter its effectiveness significantly with poly[d(A-T)] as template, within the limits studied, but with *H. cutirubrum* DNA the priming ability of the oligonucleotides decreased rapidly with increasing chain length.

Finally, the template specificity of the primed reaction was studied (Table 8). All four templates examined were effective both in the presence and absence of 1.5 M-KCl-0.6 M-NaCl. In these conditions, with the complete enzyme ($\alpha\beta$), *H. cutirubrum* DNA was a template with salt only and calf thymus DNA only in the absence of salt, whereas T_4 DNA was not accepted as a template at all (Louis & Fitt,

Table 6. *Effect of oligonucleotides on RNA synthesis catalysed by protein α alone*

Incorporation of [^{14}C]ATP into an acid-insoluble form was measured by the standard assay with protein α (40 ng) as enzyme together with the indicated oligonucleotides (5 nmol).

Oligonucleotide	ATP incorporation (nmol)	
	<i>H. cutirubrum</i> DNA	Poly[d(A-T)]
None	0	0
ApA	1.30	1.33
(Ap) ₂ A	1.15	1.18
(Ap) ₃ A	0.73	1.30
(Ap) ₄ A	0.29	1.24
(Ap) ₅ A	0.098	1.33
ApU	1.69	—
GpU	1.66	—
CpC, CpU or CpG	0	—
ApA + rifampicin (1.16 nmol)	1.28	—

Table 7. *Nearest-neighbour analysis of product of ApA-primed poly(A-U) synthesis catalysed by protein α*

A large scale (0.5 ml) standard reaction (see the Experimental section and Table 6) was done with poly[d(A-T)] as template, ApA as primer and all four nucleoside triphosphates, with [α - ^{32}P]ATP instead of [^{14}C]ATP. Incubation was at 37°C for 2 h and a sample (25 μl) was then processed in the usual way to determine total incorporation. The methods described in the preceding paper (Louis & Fitt, 1972: Table 5) were used for (i) isolation of the bulk product by phenol extraction; (ii) alkaline hydrolysis of the purified RNA; and (iii) separation, identification and radioactivity assay of the resulting 2'(3')-[^{32}P]NMP. (The protein α used had been stored for 5 months and had about 25% of its original activity.)

	(nmol)	
Total incorporation of 5'-[^{32}P]AMP units	1.61	(100%)
Recovery of 2'(3')-[^{32}P]UMP	1.42	(88.1%)
Recovery of 2'(3')-[^{32}P]AMP	0.0013	(0.1%)
Total recovery	1.421	(88.2%)

1971b, 1972). It is therefore particularly noteworthy that T₄ DNA should be an excellent template in the primer-dependent reaction at both high and low ionic strength. These results prove conclusively that the salt-dependent template specificity of the complete enzyme is determined solely by the β subunit.

Discussion

Our studies (Louis & Fitt, 1972) of the enzymic properties of the complete *H. cutirubrum* DNA-dependent RNA polymerase have shown that the

Table 8. *Lack of template specificity in the primer-dependent reaction*

Standard assays (see the Experimental section) were performed with the indicated templates (30 μg), in the presence and absence of 1.5 M-KCl–0.6 M-NaCl. Protein α (40 ng) was used as enzyme and ApA (5 nmol) as primer.

DNA	Salt	ATP incorporation (nmol)
None	+	0.00
None	—	0.00
<i>H. cutirubrum</i>	+	1.27
<i>H. cutirubrum</i>	—	1.07
T ₄ coliphage	+	1.25
T ₄ coliphage	—	1.20
Calf thymus	+	1.22
Calf thymus	—	0.98
Poly[d(A-T)]	+	1.27
Poly[d(A-T)]	—	1.20

latter resembles the much larger *E. coli* polymerase in many respects. In particular, they both recognize the same initiation signals. However, our physical studies (Louis *et al.*, 1971; Louis & Fitt, 1971b) of the two subunits α and β and of the complete enzyme, $\alpha\beta$, also show that the α and β subunits do not interact in the strong salt solutions (total salt concentration >2 M) required for maximum activity with *H. cutirubrum* DNA and most of the other natural templates tested (Louis & Fitt, 1972). The results in the present paper show that the cations Mg^{2+} and Mn^{2+} , both of which are required for activity (Louis & Fitt, 1971a), play an essential role in the formation of the $\alpha\beta$ complex and in its binding to the DNA template. It is probable that this is a sequential process in which the

$\alpha\beta$ complex must first form in the presence of Mn^{2+} , to give the active enzyme with an approx. mol. wt. 36000, and that the latter is then able to bind to the template in the presence of Mg^{2+} . This is unexpectedly different from the behaviour of the large and complex Mg^{2+} -dependent *E. coli* RNA polymerase, which does not require Mg^{2+} to bind to the template (Richardson, 1969).

E. coli RNA polymerase consists of five large subunits, $\alpha_2\beta\beta'\sigma$, and has mol.wt. 400000 (Burgess, 1969, 1971). It appears that the β' subunit is required for primary binding of the enzyme to DNA (Zillig *et al.*, 1970), that β is the rifampicin-binding site (Heil & Zillig, 1970), and that σ determines the specificity of initiation (Bautz *et al.*, 1970; Travers, 1970). The function of the two α subunits is not known nor is it clear whether the catalytic site is on one chain only or formed by a combination of subunits. In contrast, the relative simplicity of the *H. cutirubrum* DNA-dependent RNA polymerase, and the existence of the primed reaction specifically involving one subunit only, permits a clear assignment of functions to one or other of proteins α and β or to the complete enzyme, $\alpha\beta$.

First, the studies of the inhibition of the reaction by rifampicin demonstrate that the binding site for the antibiotic is on the β subunit, since only the latter can relieve inhibition when added in excess and the primed reaction with α alone is insensitive to rifampicin. Secondly, the lack of salt-dependent template specificity in the primed reaction, involving the α subunit and an oligonucleotide, is in marked contrast with the great variation in template specificity of the complete enzyme, $\alpha\beta$, with ionic strength (Louis & Fitt, 1971*b*, 1972). These results suggest that the β subunit is also responsible for specific initiation and thus combines the functions of the *E. coli* polymerase β subunit and σ -factor. Thirdly, the fact that the primed reaction is observed with protein α , but not with protein β , shows that protein α contains the catalytic site: in this connexion it would be worthwhile to study the possible catalytic activity of both single *E. coli* subunits and combinations of the latter in the presence of oligonucleotide primers. Finally, the inability of the α or β subunit alone to catalyse the formation of the first phosphodiester bond of a new RNA chain,

even though the α subunit carries the catalytic site, suggests that only the complete enzyme, $\alpha\beta$, permits the correct alignment of the terminal nucleotides in a specific initiation complex. This distribution of activities between α , β and the complete enzyme, combined with the relatively small size of the two subunits, should permit a detailed study of the mechanism and structural requirements of each individual function.

We thank the Medical Research Council of Canada for an operating grant (to P. S. F.). Requests for reprints should be addressed to P. S. F.

References

- Bautz, E. K. F., Dunn, J. J., Bautz, F. A., Schmidt, D. A. & Mazaitis, J. (1970) *Proc. Lepetit Colloquium RNA Polymerase and Transcription 1st.*, Florence (Silvestri, L., ed.), p. 90, North-Holland Publishing Co., Amsterdam
- Burgess, R. R. (1969) *J. Biol. Chem.* **244**, 6168
- Burgess, R. R. (1971) *Annu. Rev. Biochem.* **40**, 711
- Cassani, G., Burgess, R. R. & Goodman, H. M. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 47
- Dunn, J. J., Bautz, F. A. & Bautz, E. K. F. (1971) *Nature New Biol. (London)* **230**, 94
- Fischer, L. (1969) in *Laboratory Techniques in Biochemistry and Molecular Biology* (Work, T. S. & Work, E., eds.), vol. 1, p. 157, John Wiley and Sons, New York
- Fitt, P. S. & Louis, B. G. (1970) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **29**, 876
- Heil, A. & Zillig, W. (1970) *FEBS Lett.* **11**, 165
- Lill, H., Lill, U., Sippel, A. & Hartmann, G. (1970) *Proc. Lepetit Colloquium RNA Polymerase and Transcription 1st.*, Florence (Silvestri, L., ed.), p. 55, North-Holland Publishing Co., Amsterdam
- Louis, B. G. & Fitt, P. S. (1971*a*) *Biochem. J.* **121**, 621
- Louis, B. G. & Fitt, P. S. (1971*b*) *FEBS Lett.* **14**, 143
- Louis, B. G. & Fitt, P. S. (1971*c*) *Proc. Can. Fed.* **14**, 107
- Louis, B. G. & Fitt, P. S. (1972) *Biochem. J.* **126**, 69
- Louis, B. G., Peterkin, P. I. & Fitt, P. S. (1971) *Biochem. J.* **121**, 635
- Richardson, J. P. (1969) *Progr. Nucl. Acid Res. Mol. Biol.* **9**, 75
- Travers, A. A. (1970) *Nature (London)* **225**, 1009
- Warburg, O. & Christian, W. (1942) *Biochem. Z.* **310**, 384
- Zillig, W., Zechel, K., Rabussay, D., Schachner, M., Sethi, V. S., Palm, P., Heil, A. & Seifert, W. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 47