Properties of a *Bacillus subtilis* strain lacking DNA polymerase I

Giuseppe Villani, Umberto Canosi, Adriana Fortunato, Giorgio Mazza,
Mario Polsinelli and Arturo Falaschi

Laboratorio di Genetica Biochimica ed Evoluzionistica del Consiglio Nazionale delle
Ricerche and Istituto di Genetica dell'Università, Via S. Epifanio, 14, 2700 Pavia, Italy

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ABSTRACT

We have isolated a mutant of *Bacillus subtilis* deficient in DNA polymerase I, denominated polA42, which shows a reduced ability to repair the damage to DNA by UV radiation, MMS and mitomycin C; the ability to perform recombination is not appreciably impaired. DEAE cellulose chromatography allows the separation of polymerases I and II from the parental strain; a simple procedure is also described which allows to separate rapidly the polymerases II and III of the mutant strain. The three separated polymerases have similar catalytic properties but they can be distinguished for their sensitivity to inhibitors: PCMB inhibits polymerases II and III but not polymerase I; HPURa inhibits only polymerase III. All three enzymes are unaffected by nalidixate. The DNA synthesis occurring in cells of the polA42 strain permeabilized with toluene is inhibited by nalidixate, whereas the synthesis occurring in polA⁺ toluenized cells is unaffected by the drug. The polA gene has been mapped by transduction and localized between the phe₁₂ and arg₃ genes.

INTRODUCTION

A number of mutants of *Bacillus subtilis* deficient in the repair of damage by UV^{*} or by MMS have been isolated in our laboratory in the last few years¹. They have been characterized also for the ability to be transformed, transfected and transduced, for the ability to take up DNA, for the resistance to mitomycin C and for the reactivation of UV irradiated phage. One of the mutants sensitive to UV and MMS turned out to be deficient in

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Abbreviations and symbols:

UV, ultraviolet radiation; MMS, methyl-methane-sulfonate; his, trp, tyr, aro, phe, arg, requirements for histidine, tryptophane, tyrosine, shikimic acid, phenylalanine, arginine, respectively; ery, resistance to erythromycin; polA, deficiency in DNA polymerase I; Pol I, Pol II, Pol III, the enzymes DNA polymerase I, II and III; m.i.c., minimum inhibitory concentration; pfu, plaque forming units; HPURa, 6(p-hydroxyphenylazo) uracil; DTT, dithio-threitol; PCMB, p-chloromercuribenzoate.

DNA polymerase. The enzymological characterization of the mutant and of the wild type demonstrates in agreement with other authors^{2,3,4}, that also in *B. subtilis* three DNA polymerases can be observed, one of which is absent in the particular mutant denominated polA42.

In this paper we report the properties of the mutant, the demonstration of the existence of the three different polymerases, and the location of the gene for DNA polymerase I on the *B. subtilis* map.

MATERIALS AND METHODS

Bacterial strains and bacteriophages - The origin and the description of the strains used in this work are reported in Table I. The transducing phage PBS1 was received from Dr. I.D. Goldberg. For transfection experiments and for the determination of host cell reactivation SPP1 phage was used⁵.

Table I
List of bacterial strains used

Strain	Genotype	Origin
PB 202	<u>his</u> ₂ , <u>trp</u> ₂ , <u>tyr</u> ₁ , <u>aro</u> ₂	SB 202 (J. Lederberg)
PB 1642	<u>his</u> ₂ , <u>trp</u> ₂ , <u>tyr</u> ₁ , <u>aro</u> ₂ , <u>polA42</u>	PB 202 treated with nitrosoguanidine
PB 1644	<u>his</u> ₂ , <u>trp</u> ₂ , <u>phe</u> ₁	SB 1058 (A. T. Ganesan ⁶)
PB 1650	<u>his</u> ₂ , <u>trp</u> ₂ , <u>phe</u> ₁ , <u>polA</u> 5	SB 1060 (A. T. Ganesan ⁶)
PB 3372	<u>phe</u> ₁₂ , <u>argA</u> ₃ , <u>ery</u>	BC 26 (J. Copeland)

Culture media - Spizizen minimal medium⁷ was used to prepare competent cells. Medium Y⁸ was used for PBS1 assays. Penassay broth (antibiotic medium n°3 Difco) and Tryptose Blood Agar Base (Difco) were used in transduction experiments. The minimal medium of Davis and Mingioli⁹ was used for selection of transformants. The PS medium⁵ and TY medium¹⁰ were used for the production of SPP1 phage lysates and for counting infective centres, respectively. Nutrient agar (Difco) was used

to determine cell titre and UV or drug sensitivity. For the enzyme preparations, the bacteria were grown in Penassay.

Reagents - MMS was purchased from Eastman Organic Chemicals Corp., Rochester, N.Y.; mytomycin C from Schuchardt Corp., München; nalidixic acid and PCMB from Mann Research Laboratories, N.Y.; HPUra was obtained from Dr. B.W. Langley, I.C.I. and the solutions were reduced according to Gass *et al.*¹¹; alumina A305, from Alcoa Chemicals Corp.; DTT from Miles Corp., Kankakee; glycyl-glycine and DEAE cellulose (medium mesh, 0.84 meq/g) from Sigma Corp., St. Louis; Sepharose 4B, from Pharmacia Corp., Uppsala.

Measurement of sensitivity to MMS and mytomycin C - MMS and mytomycin C sensitivity were tested on nutrient agar plates containing increasing concentrations of the indicated chemical, added to melted nutrient agar cooled at 45°. The sensitivity was determined as the m.i.c., after 20 hrs of incubation at 37°.

UV irradiation and host cell reactivation - A Philips 15 watt germicidal lamp was used as source of UV light.

For the survival curves, competent cells were prepared and diluted to 1×10^8 cells/ml in Spizizen minimal medium; 1 ml samples placed in 9 cm diameter Petri dishes were irradiated while gently shaken. The irradiated samples were plated on nutrient agar to measure viability.

For the measurement of host cell reactivation, 5 ml samples containing 1×10^8 pfu/ml of SPP1 in 0.01 M Tris pH 7 and 0.01 M $MgCl_2$ were irradiated while shaking, in 9 cm diameter dishes with different doses of UV. Infective centres were scored using spores plated by the soft agar technique. All the strains used in this work showed the same plating efficiency with unirradiated phage.

Transformation, transfection and transduction - Competent cells were prepared as described by Stewart¹². The transformation and transfection procedures were those described by Mazza *et al.*¹³. Transformation was performed with 0.1 µg/ml of prototroph DNA (unless otherwise indi-

cated), transfection with 0.1 µg/ml of SPP1 DNA. Transduction with PBS1 phage was performed according to Hoch *et al.*¹⁴.

DNA uptake - The ability of competent cells to incorporate radioactive DNA was determined as reported by Mazza *et al.*¹³.

Enzyme assays - DNA polymerase was assayed according to Okazaki and Kornberg¹⁵ using activated calf thymus DNA¹⁶, unless otherwise stated. Proteins were assayed by the procedure of Lowry *et al.*¹⁷.

Preparations of crude extracts - Cultures of the strains to be tested for enzyme assays or enzyme purification were grown to the middle of the log phase (140 Klett units, green filter); the cells were then collected by centrifugation, washed with saline and stored as a frozen paste. For the assays, 2 g aliquots were thawed in a mortar and 5 g of alumina were added in small aliquots; the cells were extracted by grinding, and collected with 4 ml of glycyl-glycine buffer 0.05M, pH 7.0, 10mM DTT; after centrifugation at 10.000 x g for 20 min., the supernatant was collected. For the enzymic purification, the extraction procedure was the same but scaled up proportionally to the amount of cells used.

RESULTS

Sensitivity to UV - Fig. 1 reports the survival of cells of the parental and mutant strains after UV irradiation. The mutant is markedly more sensitive: at 380 erg/mm² the difference in survival is of three orders of magnitude. The damage in repair ability is of the order of that reported for the *polA5* mutant by Laipis and Ganesan⁶. Also the ability to reactivate UV irradiated phage is significantly reduced; Fig. 2 reports the data for SPP1 phage, which are similar to the data reported by Laipis and Ganesan for the *polA5* strain and SPO1 phage⁶.

Sensitivity to MMS and mitomycin C - The mutant is also more sensitive than the parental strain to these two drugs; whereas the m.i.c. for MMS of the parental strain PB202 is of 600 µg/ml, it is of 350 µg/ml for strain *polA42*; for the parental strain and *polA5* mutant isolated by Laipis and Ganesan⁶, the corresponding m.i.c.'s were 500 and 250

Fig. 1 - Effect of UV on viability

The bacterial cultures were irradiated for the indicated doses and the ability to form colonies was determined, according to the procedures reported in "Materials and Methods".

Fig. 2 - Reactivation of UV irradiated SPP1 phage

The plating efficiencies of irradiated suspensions of SPP1 phage were determined by the procedure reported in "Materials and Methods".

$\mu\text{g/ml}$ respectively. Both parental strains have a m.i.c. for mitomycin of $0.05 \mu\text{g/ml}$, whereas polA42 and polA5 have a m.i.c. of $0.04 \mu\text{g/ml}$. The difference in sensitivity between mutant and parental strains is small for both drugs but certainly significant. The drug sensitivity of the two independently isolated polA strains is similar. The step deficient in the mutants seems therefore common to the process of repair from the damage caused by all the three agents studied. Is it common also to the recombination process?

Recombination properties of the mutant - These were determined by measuring the ability of the parental and mutant strains to be transformed, transfected and transduced (a recombinational event is necessary to give origin to an infective centre in SPP1 transfection¹⁸). Transformation, transfection and transduction in the polA42 strain were respectively, 71%, 67% and 125% of the values of the parental strain. All three processes are thus essentially unaffected; the small reproducible reduction observed for transformation and transfection is probably a consequence of the reduced ability to take up DNA; in fact, this latter property is also reduced in the mutant to 70% of the parental strain. A recombinational event in which the DNA uptake process is not involved, i.e. transduction with phage PBS1, is not decreased in the mutant. We can conclude that the mutation does not affect recombination of DNA molecules inside the cell, i.e. the integration of relatively small DNA pieces (transformation), or of large DNA fragments (transduction).

Levels of enzymes acting on DNA - When we measured the DNA polymerase level using activated calf thymus DNA the activity was of 0.09 u/mg in the polA42 mutant whereas it was of approximately 0.5 u/mg in the parental strain and in a number of mutants defective in repair or recombination¹. A change in template gave more pronounced differences: with activated B. subtilis DNA the activity of the extracts of polA42 was only 5% of that of the parental. We assayed also crude extracts of the mutant and of the parental for a number of other enzymes of DNA metabolism; these were: ATP dependent DNase; a DNase specific for

single-stranded DNA; an endonuclease acting on double stranded DNA; DNA ligase (data to be published elsewhere). All these enzymes are present in the mutant at the same level as in the parental strain.

Purification of DNA polymerase - In a previous paper¹ we reported how a purification procedure including streptomycin precipitation, autolysis, ammonium sulfate precipitation and DEAE cellulose chromatography, gave two peaks of polymerase from the parental strain, one eluting at approximately 0.1 M potassium phosphate buffer, pH 7.4, the other around 0.2 M. The latter peak corresponds to the polymerase described by Okazaki and Kornberg¹⁵ and seems absent in the polA42 strain. Since the purification involved marked losses of activity and an autolysis step where proteolytic degradation of the enzyme could take place, we have later devised a new procedure of purification, whereby the nucleic acids are removed by the use of a Sepharose column.

Table II
Purification of DNA polymerases I and II
from the parental and mutant strains

Step	Parental		Mutant	
	Total units	u/mg	Total units	u/mg
Crude extract	862	0.44	257	0.22
Streptomycin precipitate	416	1.98	104	0.43
Sepharose eluate	262		42	
(NH ₄) ₂ SO ₄ precipitate	239	2.11	34	0.71
DEAE cellulose eluate A	15	1.30	8.1	1.20
DEAE cellulose eluate B	53	2.60	-	-

Table II summarizes the results of such a purification from 30 g of parental and 20 g of mutant cells. The crude extract was made as reported

in Materials and Methods. It was then diluted with enough extraction buffer to bring the protein concentration to 16 mg/ml and DTT was added to 10 mM; 0.25 volumes of a fresh 5% solution (w/v) of streptomycin sulfate were added with stirring. The suspension was centrifuged at $10.000 \times g$ for 15 min. The precipitate was suspended in 20 ml of 1 M Tris-HCl pH 7.5, 0.5 M NaCl, 10 mM DTT. The streptomycin precipitate was then placed on a Sepharose 4B column 2.3 cm diameter, 45 cm high, equilibrated with 1 M Tris-HCl, pH 7.5, 0.5 M NaCl; the elution was performed with the same buffer containing also 10 mM DTT and 1 mM EDTA; 10 ml fractions were taken and assayed for DNA polymerase and protein concentration; the polymerase appeared between 60 and 150 ml. To the pools of the Sepharose eluates we added ammonium sulfate up to 0.6 g per ml. The precipitate was collected and redissolved in 0.05 M potassium phosphate buffer, pH 7.4, 10 mM DTT, 2 mM EDTA, dialysed vs. the same buffer and adsorbed on DEAE cellulose columns of $4.5 \text{ cm}^2 \times 4 \text{ cm}$ for the parental strain, and of $2.8 \text{ cm}^2 \times 4$ for the polA42 strain; the columns were eluted with a gradient between 0.05 and 0.5 M potassium phosphate, pH 7.4, containing 10 mM DTT and 2 mM EDTA (200 ml of gradient for the parental, 100 ml of gradient for the mutant). The results of the chromatography are reported in Fig. 3. The enzyme from the parental strain yields two distinct peaks, again, as in the previous procedure, eluted at 0.1 M and 0.2 M potassium phosphate approximately. The mutant instead yields only one peak, at the lower ionic strength.

Rechromatography of the separated peaks - Each peak of the DEAE gradient was pooled as indicated in Fig. 3 and analysed in a second column similar to the previous one; this was done to rule out artifacts of the DEAE chromatography due to the possible presence of nucleic acids in the enzyme fraction. As the lower portion of Fig. 3 shows, each peak chromatographs true at the same ionic strength as in the previous column. It is confirmed then that we are dealing with two molecular species, one of which is absent in the polA42 mutant. The two enzymes can be iden-

Fig. 3 - Chromatography of DNA polymerase on DEAE cellulose
For the procedure see text. The fractions of the first chromatography were pooled as indicated in the upper portion of the figure, and rechromatographed as described in the text.

tified as polymerase I (for the peak eluted at high ionic strength) and polymerase II (for the other one), in agreement with the order of discovery and the terminology used by the other authors. Are the catalytical properties of the two polymerases ostensibly different?

Effect of different templates on the activity of the DNA polymerases -

The change in template affects markedly the initial rate of synthesis with the partially purified enzymes. Native nicked calf thymus DNA, B. subtilis DNA, poly(dAT):(dAT), poly(dA):poly(dT) were all good templates, and no particular difference is observed neither among them nor between the two polymerases for the initial rate of polymerization. A pronounced difference is seen when nicked denatured DNA is used as template; denaturation of the template reduces to one tenth the activity of Pol I of the parental strain, whereas it brings to less than 1% the activity of Pol II whether from the parental or the mutant strain. An analogous difference in response is observed when high molecular weight native SPP1 DNA (a linear duplex free of endonucleolytic breaks) is used as template. Pol I, with this intact template, gives substantial activity of the order of 10% of the optimal observed with nicked native DNA, whereas Pol II activity is not detectable, and less than 1% of the optimal. The introduction of an increasing number of breaks with pancreatic DNase improves the rate of all the enzymes, but whereas a few breaks are sufficient to raise the activity of Pol I (so that after 10 breaks, 70% of the maximum activity is observed), over 40 breaks are necessary to bring Pol II to 70% of the optimum. It is still possible that an endonuclease present in Pol I preparations is responsible for the lesser demand of nicks for the optimal activity of the enzyme.

Purification of polymerase III - As reported by other authors^{2, 3, 4, 19}, a third polymerase can be isolated from the polA mutants. The enzyme is masked by the excess of Pol I in the DEAE column eluate of the parental strain; also in the extracts of PolA42 strain, a peak of activity corresponding to what was described by Gass and Cozzarelli² as Pol III, can be observed only if precautions are taken to avoid inactivation of the

enzyme, in view of its pronounced instability. In order to observe polymerase III, polA42 cells were grown until the middle of the log phase, collected and washed with saline; 9 g of fresh cells were immediately extracted with 22.5 g of alumina and 20 ml of 0.4 M potassium phosphate buffer pH 7.4, 2 mM DTT, 1 mM EDTA. 20 ml of supernatant were obtained after centrifugation at $10.000 \times g$ for 15'; these were placed immediately on a DEAE cellulose column ($2.5 \text{ cm}^2 \times 4 \text{ cm}$) equilibrated with the same buffer and two column volumes of washing with the same buffer were collected together with the filtered extract; the filtrate was dialyzed vs 0.01 M potassium phosphate pH 7.4, 2 mM DTT, 1 mM EDTA, 20% glycerol. The dialyzed fraction was centrifuged for 3 hrs at 36.000 rpm in rotor 40 of the Spinco ultracentrifuge; the supernatant (27 ml) was added on a DEAE cellulose column ($7.7 \text{ cm}^2 \times 13 \text{ cm}$) equilibrated with the same buffer. This column was washed with 200 ml of the equilibrating buffer; a linear gradient from 0.01 M to 0.5 M potassium phosphate pH 7.4 (containing 2 mM DTT, 1 mM EDTA, 20% glycerol) was applied; Pol II was collected in the wash; Pol III eluted around 0.28 M potassium phosphate. The amounts of units of Pol II and Pol III thus separated are 15 and 2 (starting from a total of 20 units in the crude extracts).

Properties of the separated polymerases - Pol III has general properties similar to those of Pol II, as far as the response to different templates is concerned. The three polymerases can be distinguished from each other on the basis of their sensitivity to inhibitors. As reported in Table III, and in agreement with the observations of Gass and Cozzarelli², Pol II and III are inhibited by thiol group reagents; Pol III only is inhibited by HPUra, an inhibitor of replicative DNA synthesis²⁰. None of the polymerases is affected by nalidixate: this is as expected, since other data²¹ indicate that some component other than the DNA polymerase present in the replication apparatus must be the target of this inhibitor.

Other properties of the mutant - The absence of Pol I allows an easier

Table III

Sensitivity of *B. subtilis* polymerases to inhibitors

Inhibitor	Concentration	% residual activity		
		Pol I	Pol II	Pol III
PCMB	1.0 mM	89	<0.5	<0.5
Nalidixate	0.86 mM	100	115	99
Nalidixate	2.80 mM			115
HPUra	0.05 mM			22
HPUra	0.10 mM			13
HPUra	0.20 mM	120	106	10

preparation of subcellular DNA synthesizing systems in which the contribution of repair-type synthesis is reduced. Table IV reports the rates of DNA synthesis after toluenization of cells from the parental and from the mutant strain. The *polA42* strain gives a lower rate of overall incorporation than the parental, but it is inhibited both by HPUra and by nalidixate, the latter a very specific inhibitor of replicative synthesis. The synthesis observed in the parental cells is higher, and partially HPUra sensitive, but is less ATP dependent and totally insensitive to nalidixate. Thus, the toluenized *polA42* cells satisfy a very stringent criterion to define how physiological a subcellular DNA replicating system is, i.e. the sensitivity to nalidixate²¹. The lack of sensitivity to this drug of the toluenized *polA*⁺ cells indicates that a good fraction of the incorporation observed with this strain is of the repair type. The partial sensitivity of the toluenized *polA*⁺ cells to HPUra is not in contrast with this conclusion, since Pol III is also involved in the repair of UV induced damage, at least in *E. coli*²², so that an inhibition of this en-

Table IV

DNA synthesis rate in toluenized cells of the parental and mutant strains

The toluenization procedure and the composition of the reaction mixture were as reported by Brown²³. Each assay contained approximately 1.2×10^8 cells in 0.25 ml; the radioactive precursor was ^3H dATP, with a specific activity of 116 cpm/pmol. The incubation was for 20 min at 30°.

Strain	Condition	pmol dAMP incorporated by 10^8 cells in 20 min	% residual rate
parental	Complete system	25.0	-
	- ATP	12.2	49
	+ 0.2 mM HPURa	10.0	40
	+ 0.86 mM nalidixate	24.0	96
<u>polA42</u>	Complete system	11.4	-
	- ATP	3.0	26
	+ 0.2 mM HPURa	3.8	33
	+ 0.86 mM nalidixate	5.8	51

zyme does not suffice to demonstrate that one is certainly observing a physiological replicative process. The possibility that the polA⁺ system is insensitive to nalidixate because the toluenization has caused the loss of some hypothetical enzyme modifying the drug into a truly active form, is made unlikely by the fact that similarly treated polA⁻ cells are sensitive to the drug.

Mapping of the polA gene - In a previous paper¹, we reported that the polA42 mutation is located between the argA₃ and phe₁₂ markers. This has been confirmed by a more detailed mapping analysis. Also the polA5 mutant described by Laipis and Ganesan⁶, maps in the same region (Fig. 4). A more detailed genetic analysis demonstrates that the two polA mutants bear different closely linked mutations; Table V reports the results of a transformation experiment: DNA from strain polA42 is able to transform polA5 competent cells to polA⁺ phenotype.

Fig. 4

Map of polA42 and polA5 mutations obtained by PBS1 transduction crosses
 Numbers represent recombination values (100 minus per cent of cotransfer).

Table V

Transformation between DNA polymerase defective mutants

The procedure was as described in "Materials and Methods" but with 10 µg/ml of DNA. The polA⁺ transformants were selected as colonies resistant to 500 µg/ml of MMS after 48 hrs of incubation.

Recipient strain	Donor DNA	Frequency of <u>polA</u> ⁺ transformants
<u>polA5</u>	<u>polA</u> ⁺ (PB202)	9.1×10^{-6}
"	<u>polA42</u>	7.7×10^{-7}
"	<u>polA5</u>	$<1.5 \times 10^{-9}$

The low efficiency of transformation (8% of the control) indicates that the two mutations are in the same cistron.

The order of the two PolA mutations shown in Fig. 4 has been confirmed also by reciprocal three point crosses by means of PBS1 transduction.

DISCUSSION

We have reported an independent isolation of a Pol I deficient mutant of B. subtilis. In analogy with E. coli, the absence of the enzyme causes a profound decrease in the ability to repair UV and MMS induced

damage to DNA; The recombination properties are not affected. Thus, we can assign to Pol I, like in E. coli, an essential role only in repair processes. Nothing can be said about the function of Pol II. Pol III seems directly involved in the replication process, judging from the specific inhibition by HPURA that was reported also by other authors^{2, 4, 19}. Furthermore, Cozzarelli has shown that HPURA resistant strains have a HPURA resistant Pol III¹¹, demonstrating the direct involvement of this enzyme in replicative synthesis; also, a mutation of the gene for Pol III gives rise to a mutagenic polymerase²⁴. Qualitatively the situation for the DNA polymerases seems very similar in the two bacterial species.

REFERENCES

- 1 Polsinelli, M., Mazza, G., Canosi, U. and Falaschi, A. (1973) in Bacterial Transformation, pp. 27-44, Academic Press, New York
- 2 Gass, K. B. and Cozzarelli, N. R. (1973) in Methods in Enzymology, in press
- 3 Ganesan, A. T., Yehle, C. O. and Yu, C. C. (1973) Biochem. Biophys. Res. Commun. 50, 155-163
- 4 Bazill, G. W., Gross, J. D. (1972) Nature New Biology 240, 82-83
- 5 Riva, S., Polsinelli, M. and Falaschi, A. (1965) J. Mol. Biol. 35, 347-356
- 6 Laipis, P. J. and Ganesan, A. T. (1972) J. biol. Chem. 247, 5867-5871
- 7 Spizizen, J. (1958) Proc. Nat. Acad. Sci. USA 44, 1072-1078
- 8 Yamagishi, H., Takahashi, I. (1968) Virology 36, 639-645
- 9 Davis, B. D. and Mingioli, E. S. (1950) J. Bacteriol. 60, 17-25
- 10 Biswal, N., Kleinschmidt, A. K., Spatz, H. C. and Trautner, T. A. (1967) Molec. gen. Genet. 100, 39-55
- 11 Gass, K. B., Low, R. L. and Cozzarelli, R. N. (1973) Proc. Nat. Acad. Sci. USA 70, 103-107
- 12 Stewart, C. R. (1969) J. Bacteriol. 98, 1239-1247
- 13 Mazza, G., Eisenstark, H. M., Serra, M. C. and Polsinelli, M. (1972) Molec. gen. Genet. 115, 73-79
- 14 Hoch, J. A., Barat, M. and Anagnostopoulos, C. (1967) J. Bacteriol. 93, 1925-1937
- 15 Okazaki, T. and Kornberg, A. (1964) J. biol. Chem. 239, 259-268
- 16 Richardson, C. C., Schildkraut, C. L., Aposhian, H. V. and Kornberg, A. (1964) J. biol. Chem. 239, 222-232
- 17 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. biol. Chem. 193, 265-275
- 18 Mazza, G., Galizzi, A., Minghetti, A. and Siccardi, A. (1973) Antimicrob. Ag. Chemother. 3, 384-391
- 19 Neville, M. N. and Brown, N. C. (1972) Nature New Biology 240, 80-82

- 20 Brown, N. C. (1971) J. Mol. Biol. 59, 1-16
- 21 Pedrini, A. M. , Geroldi, D. , Siccardi, A. and Falaschi, A. (1971) Eur. J. Biochem. 25, 359-365
- 22 Youngs, D. A. and Smith, K. C. (1973) Nature New Biology 244, 240-241
- 23 Brown, N. C. (1972) Biochim. biophys. Acta 281, 202-215
- 24 Bazill, G. W. and Gross, J. D. (1973) Nature New Biology 243, 241-243