A technique for the isolation of mutants of *Escherichia coli* affected in degradation of cellular RNA

Thomas G. Kinscherf, Yuan Fon Lee and David Apirion

Department of Microbiology, Division of Biology and Biomedical Sciences,
Washington University School of Medicine, St. Louis, Missouri 63110, USA

Received 7 August 1974

ABSTRACT Using a semiautomatic technique for handling large numbers of *Escherichia coli* colonies, mutants that fail to digest their cellular RNA were isolated. This was achieved by using multiwell plates where each colony is cloned in an individual well.

Cells labeled with a radioactive RNA precursor were starved for a carbon source at a high temperature. In order to assess whether or not degradation of cellular RNA took place, aliquots of each culture were subjected to autoradiography. A number of mutants defective in decay of RNA were isolated. One of them was characterized, and was found to be deficient specifically in the enzyme polynucleotide phosphorylase. Experiments carried out with this strain indicate that this enzyme participates in the degradation of "stable" RNA during carbon starvation.

Ribosomal RNA and tRNA are stable during exponential growth in bacteria (1). When bacteria are starved for essential nutrients, however, extensive degradation of these molecules takes place (2-4). Since microorganisms in nature grow exponentially for relatively short periods, and since enteric bacteria do not possess any spores or other durable forms, these processes of degradation of macromolecules are of primary biological importance.

Studying the process of rRNA decay during carbon starvation (5,6) demonstrated that the enzymes RNase II and PNPase participate in this degradation. In order to get a better insight into this phenomenon, we decided to try to isolate mutants in which the decay of cellular RNA is altered. In order to accomplish it, we adapted a technique developed by Milcarek and Weiss (7) for the isolation of DNase III⁻ mutants.

We shall describe here this technique and the characterization of one of the mutants isolated using it. This mutant is altered in the enzyme polynucleotide phosphorylase (PNPase).

MATERIALS AND METHODS

Strains. All strains used are described in Fig. 1, with the exception of N1305 (his⁻, leu⁻, argG⁻, met⁻, str⁻).

Media. Rich medium contains 8 g of Difco nutrient broth and 8 g Difco casamino acids per liter. Minimal medium was previously described (8). Starvation

Fig. 1: Genology of the strains with altered PHPase enzyme. Straight lines designate derivation by mutation, NG, N-methyl-N'-nitrosoguanidine treatment. Arrows designate crosses, double arrows lead from donors to recipients, single arrows from recipients to transductants. A cross designates a mating, with triple arrows leading to the progeny strain. Gene symbols: met, tyr, thr, leu, requirements for L-methionine, L-tyrosine, L-threonine, and L-leucine, respectively. rns, rne, and pnp, ribonuclease I, ribonuclease II, and polynucleotide phosphorylase, respectively; str, resistance to streptomycin; ts, temperature sensitivity; gll, growth inhibition by leucine. The isolation of strain D10 was described by Gesteland (13). Strain AB313 is an Hfr strain (22). Double underlinings designate the first occurrence of new mutations.

medium was like the minimal medium but devoid of glucose and was supplemented by 50 µg/ml uracil. Low phosphate medium resembled minimal medium. It contained 20 µg/ml phosphate and 0.2% casamino acids (Difco) and was buffered with 0.05 M Tris-HCl (pH 7.4).

Crosses. Prolonged matings and transduction experiments were carried out as described previously (9).

Mutagenesis. Cells were grown overnight, inoculated into 4.5 ml of rich medium, and grown with aeration to early log phase (A_{560} of 0.1 to 0.2) at 30° or 37°C. N-methyl-N'-nitro-N-nitroso guanidine (nitrosoguanidine) was added to a final concentration of 30 µg/ml, and the culture was further incubated at the growth temperature for 40 min. After centrifugation at room temperature, the supernatant was discarded, and the cell pellet was resuspended in 25 ml of rich medium, grown overnight with aeration at 30°C, and plated as necessary.

Preparation of cell extracts. Cells were suspended in TM4E buffer (0.01 M Tris-HCl, pH 7.5, 0.1 mM magnesium acetate, 1 mM dithioerythritol), approximately 4 ml per gram wet weight of cells, and subjected to high frequency sonic vibration at 0°C with a Brownwill Biosonic, small probe for 15s (250 watts). The broken cell suspension was centrifuged for 30 min at 10,000 x g, and the pellet discarded; the supernatant is referred to as the extract.

PNPase assay. This was modified from a previously described method (10). The assay measures the formation of acid-insoluble [^3H]-labeled poly(A). The reaction was started by the addition of the assay mix to an appropriate concentration of the cell extract. The mix contained in 0.1 ml: 75 mM Tris-HCl, pH 8.0; 0.66 mM MgCl_2 ; 2 mM ADI (Sigma, grade 1); ethylenediamine tetraacetic acid 0.5 mM; 0.05 µCi [^3H] ADP (18 Ci/mmol Schwarz/Mann); 20 µg of cell extract protein. After an incubation period of one hour at 30°C, the reaction was stopped with the addition of 50 µl carrier RNA (5 mg/ml yeast RNA, 0.1 M Tris-HCl, pH 7.5, 0.6 M NaCl) and 1.0 ml of 5% trichloroacetic acid (TCA). The mixture was then filtered through a glass fiber filter,

washed three times with 5% TCA, and the filters were placed in scintillation vials with 5 ml of a toluene-based scintillation fluid and the radioactivity was determined using a liquid scintillation counter (Packard Tri-carb spectrometer, counting efficiency 43% for ^3H). A blank containing no enzyme was run with each assay, the acid-insoluble counts (70-150 cpm) being subtracted from each sample.

Analysis of pre-existing RNA during starvation

Bacterial growth, labeling and RNA extraction. Cells were grown for three generations in low phosphate medium supplemented with 2 $\mu\text{Ci/ml}$ [^{32}P]-orthophosphate.

When the culture attained an absorbancy (A_{560}) of 0.5, the cells were collected by centrifugation and washed at room temperature with prewarmed starvation medium.

Starved cultures were resuspended in prewarmed starvation medium, (in the same volume as they were grown in), and incubated for different time intervals at the different temperatures. At zero-time and at the end of the starvation period, 0.5 ml samples were added to 0.5 ml of 10% trichloroacetic acid and the precipitable radioactivity was determined. The percentage of total degradation for each sample was calculated. The control culture was washed at 4°C, once with tris-magnesium buffer and once with sucrose-tris-magnesium buffer, and the resulting pellet was frozen. At the end of the starvation period, the cultures were spun down in the cold and washed with tris-magnesium buffer and sucrose-tris-magnesium buffer as above. The frozen pellets (prepared from 20 ml cultures) were resuspended in 0.7 ml of sucrose-tris-magnesium buffer, (30% sucrose, 0.01 M Tris-HCl (pH 7.7), 0.01 M magnesium acetate) 250 μg lysozyme was added and the cells were mixed gently. After 5 min in the cold 20 μl diethylpyrocarbonate was added and the cells were frozen in acetone - dry ice and thawed in cold water (four times). To the extract 1.5 mg sodium deoxycholate, 25 μg DNase and 1 mg sodium dodecylsulfate were added and it was put on ice for 10 min. The extract was centrifuged for 7 min at 5,900 x g, and to the resulting supernatant ethylenediamine tetraacetic acid (1 mM) and sucrose (100 mM) and 300 μg of bromophenol blue were added and the volume was brought to one ml.

Polyacrylamide gel electrophoresis. RNA was analyzed in a slab gel system (100 x 145 x 1.5 mm). For the separation of large RNA molecules 3.2% polyacrylamide gels were used (11). Smaller RNA molecules were separated on 12% polyacrylamide gels as described by Meyhack *et al.* (11), except that the running buffer in our system was supplemented with 0.5% sodium dodecylsulfate and the gels were run at 150 V.

Approximately 5000 cpm (0.5 to 1.5 μ g RNA) were applied in each slot. Electrophoresis was stopped when the bromophenol blue marker was 1 cm from the end of the gel. Subsequently the gels were dried, marked with 32 P and exposed to x-ray films for 15 hrs.

Quantitative analysis of degradation products. The autoradiogram was superimposed on the gel and the position of the various RNA species, as visualized on the film, were marked. A whole strip corresponding to each slot was cut, and the areas containing RNA, as well as empty areas, were cut and counted in toluene-based scintillation fluid. Background values, i.e. counts in similar gel areas in which no radioactive compounds had been separated, were subtracted.

The total radioactivity (cpm) in each gel slot was taken as 100% and the amount in each area was calculated as percentage and multiplied by the percentage of degradation calculated for this sample from a curve which measured disappearance of total preexisting RNA. Each determination was an average of at least four runs (two experiments each in duplicate). The measurement of disappearance of total RNA has been also described previously (5).

RESULTS

Isolation of mutants. Mutants were isolated by adapting the technique of Milcarek and Weiss (7). Single colonies of mutagenized cells were picked up at random by a 12 prong inoculation wheel from the agar plates and inoculated, along with parental controls, into individual wells containing 0.2 ml rich medium in the multiwell trays. The trays were covered and incubated without shaking overnight at 37°C. A metal inoculator, with 96 prongs spaced to corres-

pond to the tray wells, was used to inoculate a rich medium-agar master plate (150 x 25 Petri dish) which after incubation at 30°C for 2 or 3 days was stored at 4°C, as well as a tray which contained minimal medium supplemented with 50 µg/ml L-methionine and [^{14}C] uracil 0.1 µCi/ml. The trays were left to grow overnight at 37°C. At the end of the labeling period, the trays were centrifuged for 15 min at 0°C in an IEC PR-2 centrifuge, and the radioactive supernatant was discarded. The cells were resuspended in 0.2 ml starvation medium and spun as above. After discarding the supernatant, the cells were resuspended in 0.2 ml starvation medium, and trays were covered and incubated without shaking at 45°C. After 4 to 5 hrs of starvation, 0.1 ml of 5% trichloroacetic acid was added to each well. The trays were centrifuged as before, and aliquots of approximately 25 µl were delivered to norite-impregnated filter paper (Whatman No. 3) by means of a multi-capillary transfer unit (Fig. 2): a new device not used by Milcarek and Weiss (Fig. 2). The filter papers were autoradiographed after drying (11). Colonies the supernatant of which appeared to produce light spots on the x-ray film were retested. Dark and light spots were very similar in their intensity to those shown by Milcarek and Weiss (7) in Plate 11. About 10,000 colonies were screened. After the first trial about 200 colonies were kept and subjected to a second test. From this group only 24 colonies retested as producing light spots on the x-ray film. 12 of these colonies were tested for their ability to degrade stable RNA in assays similar to those shown in Fig. 4 (left panel). Only five showed a clear decrease in their ability to degrade preexisting RNA. These five strains were further tested for various enzymatic activities. One of these strains N2303 showed a low level of activity in a PNPase assay. We shall discuss from here onward this mutant.

The parental strain used in the isolation of this strain was H7082. This is an E. coli K12 strain which lacks RNase I, and contains an altered RNase II (see Fig. 1).

Genetic analysis. Strain N2303 was compared to the parental strain phenotypically.

Fig. 2: The multicapillary transfer unit. The box is plexiglas with dimensions of 175 mm x 105 mm x 60 mm. a, base; b, multiwell tray; c, box with capillary tubes; d, vacuum seal.

It became evident that the growth of the strain in minimal medium is inhibited by L-leucine. Like the parental strain it could grow on minimal medium supplemented with methionine, but could not grow well at elevated temperature (see Table 1).

In the first step of the analysis a transduction was carried out using D10 as donor and N2303 as a recipient, and selection was carried out for the ability to grow on minimal medium in presence of L-leucine (see Fig. 1).

All the tested transductants (65 colonies) which now could grow in minimal medium in presence of leucine, still could not grow at the elevated temperatures (see Table 1). Thirty were tested for PNPase (synthetic capacity); all were negative like the recipient strain. While the parental strain N7082 synthesized about 1400 cpm worth of poly (A) (see Materials and Methods), all the transductants showed activities from 80 to 170 cpm, which was similar to the fluctuation we got in different days for assaying the parental mutant strain N2303. Thus, the lesion in PNPase and inhibition of growth by leucine were clearly caused by two separate mutations. Four of the transductants were kept, one of them was assigned the number N2304 and with this strain the analysis continued.

A second transduction was carried out. Again the donor was D10 and the recipient was N2304 (see Fig. 1). Selection was for the ability to grow on minimal medium supplemented with methionine at 43°C. Numerous transductants arose, and of these 64 were purified and tested for various growth characteristics. All of them grow better than the recipient strain N2304 on minimal medium or rich medium at the elevated temperatures (Table 1). The growth was comparable to that of the donor strain D10.

Twenty transductants were assayed for PNPase, and again all turned out to be negative comparable to the parental strains N2303 and N2304. The level of enzyme was similar to that found in the previous transduction (D10 → N2303). This experiment showed that the failure to grow at elevated temperatures was not caused by the lesion in PNPase. A number of these transductants were kept, one

Table 1. Growth Characteristics of Strains

Strain	37°C			43°C		45°C
	Rich medium	Minimal medium	Minimal medium + leucine	Rich medium	Minimal medium	Rich medium
D10	+	+	+	+	+	+
N7082	+	+	+	+	+	+
N2303	+	-+	-	+	-	-+
N2304	+	-+	+	+	-	-+
N1112	+	+	+	+	+	+

Growth tests were done by replication from a single master plate (rich medium, 37°C) and incubating at the given temperature for 48 hrs. Minimal medium was supplemented with L-methionine. The symbols indicate, +, good growth; -+, little growth; -, no growth.

of them, designated N112, was used in further experiments.

To find out whether or not this new pnp2303 mutation is allelic with the other known three alleles in the PNPase gene (12), a transduction experiment was carried out. Since the pnp locus is cotransduced with the argG locus (12), strain N112 was used as donor and strain N1305 (argG⁻) as the recipient. Selection was carried out for the ability to grow in the absence of arginine. A large number of transductants arose, and thirty of them were analyzed for PNPase activity. Twenty-four of these transductants had a low level of PNPase indistinguishable from the levels in the parental strain N112, i.e. 80% cotransduction between argG and pnp 2303, very comparable to the result of 77% obtained by Reiner (12). Therefore, it is very likely that this new pnp⁻ mutation is in the same gene as the pnp mutations isolated by Reiner (12).

Enzyme analysis. Since the technique by which the mutant strain N2303 was isolated was not a specific technique for finding strains defective in PNPase, but rather was a technique designed to isolate mutants defective in decay of 'stable' RNA during starvation, the possibility existed that more than a single enzyme was deficient in this strain. In order to test this possibility we tested strains N2303 and the parental strain N7082 for their respective levels of the enzymes ribonuclease I (13), II (14), III and PNPase. Both strains lacked RNase I and contained a thermolabile RNase II, but normal levels of RNase III (Fig.3). However, while strain N7082 had normal levels of PNPase, strains N2303, N2304 as well as strain N112, had no detectable levels of PNPase (see Fig. 3).

In Fig. 3 the synthetic capacity of PNPase was assayed. We measured also the degradative capacity of PNPase (15) in the same strains as well as in a pnp 13 strain. Again we found that while strain N7082 had normal levels of PNPase, both strains N2303 and N112 had a similarly low level of PNPase.

Degradation of RNA during carbon starvation. The mutants isolated here were

picked out because of their failure to degrade preexisting RNA. Recently the process of ribosome disintegration during carbon starvation, was analyzed in some detail (5,6). These studies showed, that during carbon starvation ribosomal subunits disintegrate by a process which involve endo and exoribonucleases. The process starts by endonucleolytic attack on rRNA, by thus far unidentified enzyme(s). The products of this endo-nucleolytic attack are RNA molecules about 4S in size. These pieces of RNA are degraded to nucleotides mainly by PNPase and also by RNase II. Therefore we studied the process of degradation of preexisting RNA during carbon starvation in strains N7082 and N1112 (See Fig. 4).

The degradation of total RNA during starvation was compared in both strains at 37°C and 50°C. At 37°C no significant difference was observed between the strains, while at 50°C strain N1112 showed less degradation of its preexisting RNA than strain N7082 (Fig. 4). (50°C was chosen as the elevated temperature, since previously at that temperature we observed clear cut differences between pnp⁺ and pnp⁻ strains, refs. 5,6).

On further analysis of the process we found that while the large rRNA molecules 23S and 16S disappeared very much similarly in both strains (Fig. 4, middle panel), strain N1112 had difficulties in removing the pieces of RNA produced by the endonucleolytic attack on the 23S and 16S RNA (right hand panel). The small pieces of rRNA accumulated in strain N1112 during the starvation are shown in Fig. 5. In previous studies, Kaplan and Apirion (6) found similar results with strain N7060 (14) which like N1112 lacks RNase I and carries an altered RNase II and PNPase.

In previous studies with strains AB301, A19, Q13, and N7060 (manuscript in preparation), a 15S-like molecule, a degradation product of 16S (d 16S) was encountered. It was suggested that RNase II is responsible for the appearance of this molecule. Here we can see (Fig. 6) that in strain N7082, where RNase II

Fig. 3: Extracts from pnp^+ and pnp^- strains were prepared and assayed for RNase III (Weatherford et al. 14) (left panel) and PNPase (right panel; synthetic assay) as described in Methods, 40 μ g protein were used for the RNase III assay and 20 μ g protein for the PNPase assay. Assays were carried out at 35°C.

Fig. 4: Disappearance of 23S and 16S RNA during carbon starvation at 50°C. [32 P] - RNA, prepared from control cultures and from cells starved for 30 min, 60 min and 120 min was separated on 3.2% polyacrylamide gels. The amounts of 23S RNA, 16S RNA and RNA smaller than 16S were determined and corrected as detailed in Materials and Methods. The data presented in the Figure are the percentages of the respective RNA species remaining in the starved cells as compared to the control. Data on 16S RNA include d16S RNA (see Fig. 6). The left panel represents solubilization of total RNA (for further details see ref. 5). The first three panels are read against the left scale and the fourth panel against the right scale.

is missing but PNPase is present, this molecule also appears. It does not appear in strain N1112, similar to the results obtained with strain N7060. Therefore it seems that this molecule can be produced by the attack of either of the enzymes ribonuclease II or PNPase on the 3' end of the 16S rRNA molecule.

DISCUSSION

The technique. The technique described here for the isolation of E. coli mutants, which was developed from the procedure described by Milcarek and Weiss (7), has obviously a great potential for the isolation of many types of mutants in bacteria or other organisms for which an obvious selection or screening technique is unavailable. While Milcarek and Weiss (7) showed how to use the multiwell plates for the isolation of mutants deficient in a specific enzyme, we demonstrated here that this technique can be used for the isolation of mutants where the substrate and enzyme are both included within the cell.

The nature of the pnp mutation. The strains described here, as well as some of the strains described by Reiner (16) show a very low level of PNPase-like activity. These strains are viable under most growth conditions. Thus the question arises, how vital is the enzyme to the bacterial cell? One possibility is that PNPase indeed exists within these mutant strains, but in such minute quantities that it goes undetected by the assay techniques. This possibility suggests that only very small amounts of PNPase are necessary to maintain cell function.

A second possibility is that there is some other enzyme in the cell that can take over PNPase's function when the enzyme is at low levels. This agent would not necessarily be active under the conditions imposed in the PNPase assay procedures and so would not be detected.

A third possibility is that there are higher levels of this enzyme in vivo than found in vitro. There are two indications that suggest that this might be the case. First, Thang et al. (10) found that strain Q13, carrying the mutation

Fig. 5: Degradation products from ribosomal RNA. RNA from strain N1112 was prepared at different times during starvation, and separated on a 12% polyacrylamide combination gel (11). The autoradiograph of this gel is pictured here.

Fig. 6: Appearance of d16S RNA. RNA from strains N7082 and N1112 was prepared at zero time and 60 minutes of starvation and separated on a 3.2% polyacrylamide gel. The autoradiograph of this gel is pictured here. The arrow points out to the d16S RNA species.

pnp13, contains quite high levels of PNPase when tested with Mn^{2+} rather than with Mg^{2+} (synthetic capacity). Second, among the pnp mutants, the strain that carries the pnp27 mutation contains a thermolabile PNPase (16, 17). When cells of such a strain are grown at 30°C or 37°C and opened, they contain about 50% of the wild type activity; this activity is thermolabile. However, when the strain is tested for degradation of its stable RNA during carbon starvation at 50°C it shows a low activity very comparable to those of strains carrying the alleles pnp7 and pnp13 (5). On the other hand at 37°C no differences between strains carrying the three pnp alleles 7, 13, and 27 and strains carrying wild type alleles are observed for degradation of stable RNA during carbon starvation. Therefore it is quite likely that the pnp7, 13, and 2303 alleles are caused by missense mutations, which reduce the stability of the enzymes upon opening of cells.

How strain N2303 might have arisen. The strain N2303 which was isolated from the parental strain N7082 contained at least three mutations (see Fig. 1 and Table 1): one causing sensitivity to growth in minimal medium in presence of leucine, the second causing inability to grow at high temperatures, and the third causing the pnp mutation. It is often argued that nitrosoguanidine causes multiple closely linked mutations, since it is affecting specifically the DNA replicating fork (18). There are two arguments against such a simple explanation. First, many workers using nitrosoguanidine employ the technique described by Adelberg, Mandel and Chen (19), which subjects cells to a relatively harsh treatment which apparently often causes multiple mutations. The technique employed by us is much gentler, subjecting exponentially growing cells to the relatively low concentration of 30 µg/ml of the drug for only 40 min. We use this technique routinely, and in some of the cases when Ts^- mutations were analyzed, a number of phenotypic differences observed between the mutants and parental strains were shown to be caused by a single point

mutation (see for instance ref. 9). Furthermore, if the three different mutations observed in strain N2303 were closely linked, we would expect that in at least one case when the genetic analysis (transductions) was carried out some linkage would be observed between the mutation transduced out and the pnp⁻ mutation. Such linkage was not observed (see Results).

The various mutations induced in strain N2303 could be unrelated, but some of our observations suggest that at least one of these mutations is physiologically related to the pnp⁻ mutation. In studies on the degradation of rRNA during carbon starvation with the different strains derived from N7082 (unpublished observations) we noticed that strain N2303 carries a factor that increased rRNA degradation. Since a pnp mutation decreases rRNA degradation during starvation, these two mutations are physiologically compensating one for the other. A similar phenomenon was found when strain AB105, which contains an altered RNase III, was analyzed (20).

The function of PNPase. This enzyme has the capacity to carry out truly reversible reactions, phosphorylisis of polyribonucleotides as well as their synthesis. The results presented here support previous observations (5,6) which indicate that this enzyme plays a role in starvation. When cells adapt to the condition of no external supplies by decaying the ribosomal RNA, this enzyme is involved in degrading the ribooligonucleotides made from the ribosomal RNA to nucleotides (see Fig. 4, right panel). Using this enzyme makes a lot of biological sense, since it transfers polynucleotides to 5'-diphosphonucleotides rather than to 5'-monophosphonucleotides, which could save the cell a rich energy bond. It should be mentioned that the results presented here cannot exclude the possibility that another mutation which is still carried in strain N1112 is causing the decrease in RNA degradation, however, previous studies with isogenic strains (see ref. 5, Fig. 3) make it rather unlikely.

On the other hand, during exponential growth the enzyme has some function (21) which cannot be its degradative capacity (17), therefore it could

be its synthetic capacity. It is not yet clear how and where this function is operative. However, it is extremely interesting if there is an enzyme that can change its physiological mode of action according to the cellular environment.

Acknowledgement. Partially supported by National Science Foundation grants GB-34158, and RAMM G1-36406.

REFERENCES

- 1 Neidhart, F.C. (1964) in Progress in Nucleic Acid Research and Molecular Biology, Academic Press, New York. pp.145-181
- 2 Mandelstam, J. and Halvorson, H. (1960) Biochim. Biophys. Acta. 40, 43-49
- 3 Kennell, D. and Koutoulas, A. (1967) J. Bacteriol. 93, 334-344
- 4 Nath, K. and Koch, A.L. (1971) J. Biol. Chem. 246, 6956-6967
- 5 Kaplan, R. and Apirion, D. (1974) J. Biol. Chem. 249, 149-151
- 6 Kaplan, R. and Apirion, D. J. Biol. Chem. (In press)
- 7 Milcarek, C. and Weiss, B. (1972) J. Mol. Biol. 68, 303-318
- 8 Apirion, D. J. Mol. Biol. 16, 285-301
- 9 Lennette, E.T. and Apirion, D. (1971) J. Bacteriol. 108, 1322-1328
- 10 Thang, M.N., Thang, D.C. and Grunberg-Manago, M. (1967) Biochem. Biophys. Res. Commun. 28, 374-384
- 11 Meyhack, B., Meyhack, I. and Apirion, D. (1973) Proc. Nat. Acad. Sci. USA 70, 156-160
- 12 Reiner, A.M. (1969) J. Bacteriol. 97, 1431-1436
- 13 Gesteland, R.F. (1966) J. Mol. Biol. 16, 67-84
- 14 Weatherford, S.C., Weisberg, L.S., Achord, D.T. and Apirion, D. (1972) Biochem. Biophys. Res. Commun. 49, 1307-1315
- 15 Chou, J.F. and Singer, M.F. (1970) J. Biol. Chem. 245, 1005-1011
- 16 Reiner, A.M. (1969) J. Bacteriol. 97, 1437-1443
- 17 Krishna, R.V., Rosen, L. and Apirion, D. (1973) Nature New Biol. 242, 18-20
- 18 Cerda-Olmedo, E., Hanawalt, P.C. and Guerola, M. (1968) J. Mol. Biol. 33, 705-719
- 19 Adelberg, E.A., Mandel, M. and Chen, G.C.C. (1965) Biochem. Biophys. Res. Commun. 18, 788-795
20. Apirion, D. and Watson, H. (1974) Molec. Gen. Genet. (In press)
- 21 Krishna, R.V. and Apirion, D. (1973) J. Bacteriol. 113, 1235-1239
- 22 Taylor, A.L. and Adelberg, E.A. (1960) Genetics 45, 1233-1243