Partial Diploids of *Escherichia coli* Carrying Normal and Mutant Alleles Affecting Oxidative Phosphorylation

By FRANK GIBSON, GRAEME B. COX, J. ALLAN DOWNIE and JUDITH RADIK

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia

(Received 29 September 1976)

A plasmid was isolated which included the region of the *Escherichia coli* chromosome carrying the known genes concerned with oxidative phosphorylation (*unc* genes). This plasmid was used to prepare partial diploids carrying normal *unc* alleles on the episome and one of the three mutant alleles (*uncA401*, *uncB402* or *unc-405*) on the chromosome. These strains were compared with segregants from which the plasmid had been lost. Dominance of either normal or mutant *unc* alleles was determined by growth on succinate, growth yields on glucose, Mg-ATPase (Mg^{2+} -stimulated adenosine triphosphatase) activity, atebirin-fluorescence quenching, ATP-dependent transhydrogenase activity and oxidative phosphorylation. In all the above tests, dominance of the normal allele was observed. However, in membranes from the diploid strains which carried a normal allele and either of the mutant alleles affecting Mg-ATPase activity (*uncA401* or *unc-405*), the energy-linked functions were only partially restored.

Mutant strains of *Escherichia coli* defective in the coupling of ATP synthesis to electron transport (*unc* mutants) have been isolated in many laboratories (see Simoni & Postma, 1975). Gene–polypeptide relationships have not as yet been established for any of the mutations so far described, and so in general it has not been possible to determine whether two different mutations occur in the same gene or in different genes. It has been possible to classify broadly *unc* mutations as either affecting the ‘extrinsic’ Mg-ATPase* or the ‘intrinsic’ membrane polypeptides by reconstitution experiments *in vitro* (see Cox & Gibson, 1974). The *uncA401* and *unc-405* alleles are examples of the former class and the *uncB402* allele is an example of the latter class (see Cox & Gibson, 1974). [The *uncB401* allele is that previously described as *uncB401* (Butlin *et al.*, 1973). The change has been made to conform with the conventions of gene nomenclature.]

The use of partial diploids of the *unc* region of the *E. coli* chromosome to determine whether mutations are in the same or different genes, or to examine whether particular mutant alleles are dominant, has not been reported. In the present paper the biochemical characteristics of mutant strains carrying the *uncA401*, *uncB402* or *unc-405* alleles on the chromosome and the corresponding normal alleles on a plasmid are described. The characteristics of these diploid strains have been compared with segregant strains from which the plasmids have been lost.

* Abbreviation: Mg-ATPase, Mg^{2+} -stimulated adenosine triphosphatase (EC 3.6.1.3).

Materials and Methods

Chemicals

Chemicals were of the highest purity available commercially and were not further purified. [^{32}P]P_i (carrier-free) was obtained from the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia, and used after appropriate dilution with carrier.

Organisms

All the bacterial strains used are derived from *E. coli* K12 and are described in Table 1.

Media and growth of organisms

The medium used was that described by Monod *et al.* (1951) as ‘medium 56’. Growth supplements were added as sterile solutions at the following final concentrations: glucose, 30 mM; thiamin hydrochloride, 0.2 μM ; 2,3-dihydroxybenzoate, 40 μM ; L-arginine hydrochloride, 0.8 mM; uracil, 0.2 mM; adenine hydrochloride, 0.15 mM; L-isoleucine, 0.3 mM; L-valine, 0.3 mM.

Cells for the preparation of membranes were grown in 14-litre New Brunswick fermenters as described by Cox *et al.* (1970).

Solid media were prepared by the addition of 2% (w/v) agar to the medium described above. For agar plates containing succinate as sole source of carbon, glucose was replaced by sodium succinate (30 mM) and, in addition to the appropriate growth requirements at the concentrations given above, the medium

Table 1. *Strains of E. coli K12 used*

The gene designations follow those of Bachmann *et al.* (1976), and the plasmid nomenclature is that of Novick *et al.* (1976).

Bacterial strain	Relevant genetic loci	Other information
AT2246	Hfr, <i>leu-13, his-29</i>	Hfr P10; source of plasmid pAN1
AN627	F ⁻ , <i>argH, ilvC, pyrE, purE, recA</i>	Isolated after mating between strains AT2246 and AN627
AN738	F'(pAN1) <i>argH, ilvC, pyrE, recA</i>	
AN346	F ⁻ , <i>ilvC, argH, pyrE, entA</i>	Isolated after transduction with strain AN249 (Cox <i>et al.</i> , 1973a) as donor, and strain AN346 as recipient.
AN718	F ⁻ , <i>uncA401, argH, pyrE, entA</i>	
AN719	F ⁻ , <i>uncB402, argH, pyrE, entA</i>	Isolated after transduction with strain AN283 (Butlin <i>et al.</i> , 1973) as donor, and strain AN346 as recipient.
AN720	F ⁻ , <i>unc-405, argH, pyrE, entA</i>	Isolated after transduction with strain AN285 (Cox <i>et al.</i> , 1974) as donor and strain AN346 as recipient.
KL163	Hfr, <i>nalA, recA</i>	Obtained from J. Pittard
AN724	F ⁻ , <i>ilvC, argH, pyrE, entA, recA</i>	Isolated after mating between strains KL163 and AN346
AN730	F ⁻ , <i>uncA401, argH, pyrE, entA, recA</i>	Isolated after mating between strains KL163 and AN718
AN727	F ⁻ , <i>uncB402, argH, pyrE, entA, recA</i>	Isolated after mating between strains KL163 and AN719
AN728	F ⁻ , <i>unc-405, argH, pyrE, entA, recA</i>	Isolated after mating between strains KL163 and AN720
Diploids		
AN760	F'(pAN1), <i>ilvC, argH, pyrE, entA, recA</i>	Isolated after mating between strains AN738 and AN724
AN739	F'(pAN1), <i>uncA401, argH, pyrE, entA, recA</i>	Isolated after mating between strains AN738 and AN730
AN740	F'(pAN1), <i>uncB402, argH, pyrE, entA, recA</i>	Isolated after mating between strains AN738 and AN727
AN741	F'(pAN1), <i>unc-405, argH, pyrE, entA, recA</i>	Isolated after mating between strains AN738 and AN728
Segregants		
AN786	F ⁻ , <i>ilvC, argH, pyrE, entA, recA</i>	Segregant isolated from strain AN760
AN756	F ⁻ , <i>uncA401, argH, pyrE, entA, recA</i>	Segregant isolated from strain AN739
AN757	F ⁻ , <i>uncB402, argH, pyrE, entA, recA</i>	Segregant isolated from strain AN740
AN758	F ⁻ , <i>unc-405, argH, pyrE, entA, recA</i>	Segregant isolated from strain AN741
Plasmid		
pAN1	<i>ilvC⁺, argH⁺, pyrE⁺, unc⁺</i>	

was supplemented with Difco acid-hydrolysed 0.06% casein.

The nutrient broth used for genetic experiments was Luria broth as described by Luria & Burrous (1957), with glucose (10 mM) included as indicated.

Growth yields were measured as described by Cox *et al.* (1970), with a Klett-Summerson colorimeter.

Genetic techniques

The technique for conjugation experiments was based on that described by Taylor & Thoman (1964), and transduction experiments, in which the generalized transducing phase P1kc was used, were carried out as described by Pittard (1965). For the isolation of the plasmid pAN1, strains AT2246 and AN627 were each grown in glucose/Luria broth to a density of about 2×10^8 cells/ml. Equal parts of the cultures were then mixed and incubated at 37°C with slow shaking. The mating mixture was sampled at 40 and 90 min by plating 0.1 ml on glucose/adenine minimal-medium plates. Control plates were inoculated with 0.1 ml of the individual cultures.

Tests for the ability of strains to transfer plasmids were carried out by cross-streaking male and female strains, grown in glucose/Luria broth to mid-exponential phase, on the appropriate medium, and observing the formation of transconjugant colonies in the sections of the streaks where the cells were mixed. Partial diploid strains were isolated by streaking out transconjugants from plate matings as above and isolating and examining four separate strains for their growth characteristics on glucose and succinate media. The four strains from each mating showed the same phenotype, and one was chosen for further study.

Segregants from partial diploids were obtained after growth in Luria broth, plating, and testing individual colonies by replica plating. Alternatively, Acridine Orange (50 µg/ml) was included in the Luria broth (pH 7.8) (Hayes, 1968).

The isolation of *recA*⁻ derivatives was carried out by mating the *recA*⁻ Hfr male, strain KL163, with the appropriate female, sampling at intervals and screening for cells resistant to both streptomycin and naladixic acid after a period of phenotypic expression.

Such strains were then tested for the presence of the *recA*⁻ allele by testing for heightened sensitivity to u.v. light. This was carried out by exposing inoculated nutrient-agar plates for 20 s to the short-wave-length u.v. lamp of a Chromato-vue cabinet (Ultra-violet Products, San Gabriel, CA, U.S.A.). The *recA*⁺ cells survived this exposure.

Preparation of cell membranes

Membranes were prepared as described previously (Cox *et al.*, 1973b). Briefly, washed cells were disintegrated by using a Sorvall Ribi cell fractionator and the 'membranes' were separated by ultracentrifugation and resuspended in a 0.1 M-Tes [*N* - tris(hydroxymethyl)methyl - 2 - aminoethane - sulphonic acid] buffer system (pH 7.0) containing magnesium acetate, sucrose and EGTA.

Protein concentrations were determined by using Folin's phenol reagent (Lowry *et al.*, 1951) with bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO, U.S.A.) as standard.

Assay of Mg-ATPase

The reaction mixture contained 0.1 M-Tris/HCl buffer (pH 9.0), 20 mM-ATP, and 10 mM-MgCl₂ in a final volume of 1 ml. The reaction was started by the addition of 5–20 µl of enzyme preparation. After incubation at 30°C in a water bath, 0.5 ml samples of the reaction mixtures were taken at 0, 3 and 6 min and added to 9.5 ml of King's (1932) reagent. After 15 min the *A*₆₆₀ was measured.

Measurement of the ATP-dependent transhydrogenase activity

The reduction of NADP⁺ was assayed by coupling the reaction to the NADPH-dependent glutathione reductase and measuring the decrease in *A*₃₄₀ (Ernster & Lee, 1967). Details of the technique used are described by Cox *et al.* (1971).

Measurement of atebrin fluorescence

Atebrin fluorescence was measured at 30°C as described by Haddock & Downie (1974) by using an Aminco-Bowman fluorimeter with the excitation wavelength set at 450 nm and emission wavelength set at 510 nm.

Oxidative phosphorylation

P/O ratios were measured as described by Cox *et al.* (1973a).

Results

Preparation of strains

A plasmid carrying the region of the chromosome between *pyrE* and *argH* (which includes the *unc* genes) was isolated after a mating between an Hfr male, with an appropriate point of origin of transfer of the *E. coli* chromosome, and a *recA*⁻ recipient carrying *pyrE*⁻, *ilvC*⁻ and *argH*⁻ alleles (see Low, 1968).

Thus the Hfr male AT2246 and the female strain AN627 (*recA*, *argH*, *ilvC*, *pyrE*, *purE*) were mated as described in the Materials and Methods section and plated on glucose/adenine minimal-medium plates. Colonies were screened by streaking on nutrient agar and testing for the presence of the *recA*⁻ allele by heightened sensitivity to u.v. light. Four of the 150 strains tested were *recA*⁻, and of these, only one (strain AN738) proved to be a male transferring *ilv*⁺, *arg*⁺, *pyr*⁺ when crossed with the female strain AN724 (*argH*, *ilvC*, *pyrE*, *entA*, *recA*). Strain AN738 was shown to contain a plasmid (designated pAN1) by the isolation of segregants which were *pyrE*⁻, *ilvC*⁻ and *argH*⁻.

Strain AN738 was then used to donate the plasmid pAN1 (*unc*⁺, *pyrE*⁺, *argH*⁺) to *argH*⁻, *pyrE*⁻, *recA*⁻ females carrying *unc*⁺, *uncA401*, *uncB402* or *unc405* alleles and the corresponding diploid strains AN760 (*unc*⁺/*unc*⁺), AN739 (*unc*⁺/*uncA401*), AN740 (*unc*⁺/*uncB402*) and AN741 (*unc*⁺/*unc405*) were isolated. Segregants from each of these strains were then isolated (see Table 1).

Growth on succinate

Mutant strains carrying *unc*⁻ alleles do not grow on a medium containing succinate as sole source of carbon. None of the segregant strains isolated, except that from the diploid carrying two *unc*⁺ alleles (strain AN786), would grow on such a medium. However, all of the diploid strains, namely AN760 (*unc*⁺/*unc*⁺), AN739 (*unc*⁺/*uncA401*), AN740 (*unc*⁺/*uncB402*) and AN741 (*unc*⁺/*unc405*), grew on the succinate medium.

Growth yields

Aerobic growth yields of the diploid and segregant strains were measured as turbidities after growth on mineral-salts media containing limiting concentrations of glucose. The segregant strains gave growth yields (Table 2) similar to those reported previously for strains carrying the *uncA401*, *unc405* and *uncB402* alleles (see Cox & Gibson, 1974). The diploid strain AN740 (*unc*⁺/*uncB402*) had a growth yield similar to that of the normal strain AN760 (*unc*⁺/*unc*⁺), but the diploids AN741 (*unc*⁺/*unc405*) and AN739 (*unc*⁺/*uncA401*) gave intermediate values (Table 2).

Table 2. *Growth yields*

Growth yields were measured as turbidities (Klett units) after growth on 5 mM-glucose.

Segregant	Growth yield (Klett units)	Diploid	Growth yield (Klett units)
AN756 (<i>uncA401</i>)	130	AN739 (<i>unc⁺/uncA401</i>)	160
AN757 (<i>uncB402</i>)	125	AN740 (<i>unc⁺/uncB402</i>)	190
AN758 (<i>unc-405</i>)	120	AN741 (<i>unc⁺/unc-405</i>)	170
AN786 (<i>unc⁺</i>)	194	AN760 (<i>unc⁺/unc⁺</i>)	195

Table 3. *Mg-ATPase, ATP-dependent transhydrogenase and P/O ratios in membranes from diploid and segregant strains*
Details are given in the Materials and Methods section. NADH was the oxidizable substrate used when determining the P/O ratios.

Strain	Mg-ATPase ($\mu\text{mol/min per mg of protein}$)	Transhydrogenase (nmol of NADPH formed/min per mg of protein)	P/O ratios ($\frac{\text{nmol of glucose 6-phosphate formed}}{\text{ng-atoms of oxygen taken up}}$)
Segregant			
AN756 (<i>uncA401</i>)	0.04	1	<0.01
AN757 (<i>uncB402</i>)	0.26	2	<0.01
AN758 (<i>unc-405</i>)	0.08	2	<0.01
AN786 (<i>unc⁺</i>)	0.25	24	0.26
Diploid			
AN739 (<i>unc⁺/uncA401</i>)	0.23	8	0.07
AN740 (<i>unc⁺/uncB402</i>)	0.41	31	0.20
AN741 (<i>unc⁺/unc-405</i>)	0.22	5	0.05
AN760 (<i>unc⁺/unc⁺</i>)	0.47	30	0.27

Mg-ATPase activity

The Mg-ATPase activities of membrane preparations from the segregant strains are consistent with previously reported data (see Cox & Gibson, 1974) in that the *uncA401* and *unc-405* alleles affect Mg-ATPase activity, whereas the *uncB402* allele does not. Membranes from the diploid strains carrying two copies of the normal genes coding for components of the Mg-ATPase have twice the Mg-ATPase activity of those carrying only one copy of the normal gene (Table 3).

ATP-dependent transhydrogenase activities

The ATP-dependent transhydrogenase activities of membrane preparations from the segregant strains are as expected (see Cox & Gibson, 1974) in that each of the three mutant alleles causes loss of activity (Table 3). Different amounts of activity were observed in the diploid strains, depending on the mutant allele present. Membranes from the diploid strain AN740 (*unc⁺/uncB402*) have ATP-dependent transhydrogenase activity essentially similar to that of membranes from the normal diploid strain (AN760) (Table 3). However, the diploid strains AN739 (*unc⁺/uncA401*) or AN741 (*unc⁺/unc-405*) have only about 20% of the activity of strains AN740 or AN760 (Table 3).

Oxidative phosphorylation

The P/O ratios obtained for membrane preparations from the various strains followed the same pattern as the ATP-dependent transhydrogenase activities and ATP-induced fluorescence quenching (see below). No detectable phosphorylation was observed by using membranes from segregant strains carrying mutations in the *unc* genes (Table 3). Membranes from the diploid strain AN740 (*unc⁺/uncB402*) gave a P/O ratio with NADH as oxidizable substrate similar to that obtained with membranes from the normal diploid strain AN760 (Table 3). However, the diploid strains carrying either the *uncA401* or *unc-405* alleles, together with a normal *unc* allele, although capable of some oxidative phosphorylation, appeared to be less efficient than the normal diploid strain (Table 3).

Atebrin-fluorescence quenching

In addition to measurement of energy-linked reactions, it is possible to monitor the energization, by ATP or electron transport, of *E. coli* membrane vesicles by measuring the quenching of atebrin fluorescence. Fig. 1 illustrates the atebrin-fluorescence quenching obtained by energization of various membrane preparations by NADH or ATP. The extent of NADH-induced fluorescence quenching is

Fig. 1. Electron-transport- and ATP-induced aetbrin-fluorescence quenching in membranes from various strains of *E. coli*. Fluorescence measurements were performed in 2.5 ml of 10 mM-Hepes [2-(*N*-2-hydroxyethyl)piperazin-*N'*-yl]ethane-sulphonic acid] buffer containing 300 mM-KCl, 5 mM-MgCl₂, about 1 mM-H₂O₂ and 250 units of catalase. Aetbrin was added to give a final concentration of 4 μ M, NADH to 1 mM, NaCN to 1 mM, ATP to 0.8 mM and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to 20 μ M. The protein concentrations were as described in Table 4. The membrane preparations used and additions made are as shown.

Table 4. Aetbrin-fluorescence quenching

Aetbrin-fluorescence quenching was measured as described in the Materials and Methods section and the legend to Fig. 1. NADH-induced quenching was calculated as a percentage decrease in relative fluorescence after the addition of NADH; ATP-induced quenching was calculated from the amount of fluorescence restored after the addition of carbonyl cyanide *m*-chlorophenylhydrazone. The protein concentrations were in the range 0.7–0.95 mg/ml.

Segregant	Quenching (%) with		Diploid	Quenching (%) with	
	NADH	ATP		NADH	ATP
AN756 (<i>uncA401</i>)	74	0	AN739 (<i>unc</i> ⁺ / <i>uncA401</i>)	75	14
AN757 (<i>uncB402</i>)	76	2	AN740 (<i>unc</i> ⁺ / <i>uncB402</i>)	79	72
AN758 (<i>unc-405</i>)	75	2	AN741 (<i>unc</i> ⁺ / <i>unc-405</i>)	75	23
AN786 (<i>unc</i> ⁺)	74	66	AN760 (<i>unc</i> ⁺ / <i>unc</i> ⁺)	73	72

similar in membranes from all strains (Table 4 and Fig. 1) and can be reversed by inhibiting respiration with cyanide (Fig. 1). However, the extents of ATP-induced fluorescence quenching vary considerably with the membrane preparations from different strains. Fig. 1(a) shows that with strain AN740 (*unc*⁺/*uncB402*) there is a considerable extent of ATP-induced fluorescence quenching, similar to that obtained with the control diploid strain AN760 (*unc*⁺/*unc*⁺) (Table 4). In contrast, no ATP-induced quenching of fluorescence was observed with membrane preparations from any of the segregant strains carrying mutant *unc* alleles (Fig. 1c; Table 4).

As illustrated in Fig. 1(b), ATP induces an intermediate extent of fluorescence quenching in mem-

branes from the diploid strain AN739 (*unc*⁺/*uncA401*), a qualitatively similar result being obtained with strain AN741 (*unc*⁺/*unc-405*) (Table 4). The extent of fluorescence quenching obtained with membrane preparations from these strains was about one-quarter of that obtained with membranes from the diploid strains AN760 (*unc*⁺/*unc*⁺) or AN740 (*unc*⁺/*uncB402*).

Discussion

The results described in the present paper indicate that the *uncB402* allele is recessive to the normal allele in that the growth on succinate, growth yield with a limiting concentration of glucose, ATP-

dependent transhydrogenase, quenching of atebriin fluorescence and oxidative phosphorylation are normal in the diploid strain AN740 (*unc⁺/uncB402*). The Mg-ATPase activity in membranes from strains AN739 (*unc⁺/uncA401*) and AN741 (*unc⁺/unc-405*) reflects the presence of the normal allele in the diploid strains and indicates that the *uncA401* and *unc-405* alleles are similarly recessive.

However, despite the fact that membranes from strains AN739 (*unc⁺/uncA401*) and AN741 (*unc⁺/unc-405*) have normal Mg-ATPase activities, they have low ATP-dependent transhydrogenase, ATP-dependent atebriin-fluorescence-quenching activities and P/O ratios. Thus in membranes from these two strains, the ability to hydrolyse ATP under the assay conditions appears to be normal, but the ATP-synthesizing ability of the ATPase complex is impaired, as is the ability to mediate other energy-linked reactions. These results indicate that some mixing of the *unc*-gene products may occur during assembly when a normal and a mutant allele are present in the same cell, and it would then follow that more than one copy of the particular polypeptides affected by the *uncA401* and *unc-405* alleles is present in a single Mg-ATPase aggregate. The intermediate growth yields obtained with the above diploids are consistent with the results obtained for the energy-linked reactions measured by using membrane preparations.

To use partial diploids to determine whether two mutations are affecting the same or different genes and these mutations affect components of a complex structure such as the Mg-ATPase, it is necessary to demonstrate that polypeptides formed from both gene copies are mixed during assembly of the final protein aggregate. If the Mg-ATPase complex is built up in some highly co-ordinated process in which the newly formed polypeptides are immediately integrated into the structure of the complex, then in a diploid containing two different *unc* mutations affecting Mg-ATPase two types of mutant Mg-ATPase could be formed. Complementation would then not occur and the incorrect conclusion would be drawn that the two mutations affected the same polypeptide. The present results suggest that mixing of gene products may occur and that a genetic approach using partial diploids, in which one

mutant *unc* allele is on the plasmid and the other on the chromosome, should give information as to the number of polypeptides required for a functional oxidative-phosphorylation system.

We thank Mr. B. Webb for skilled technical assistance and Ms. B. Homer and Mr. D. Abigail for assistance in the growth of cells and preparations of membranes.

References

- Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40**, 116–167
- Butlin, J. D., Cox, G. B. & Gibson, F. (1973) *Biochim. Biophys. Acta* **292**, 366–375
- Cox, G. B. & Gibson, F. (1974) *Biochim. Biophys. Acta* **346**, 1–25
- Cox, G. B., Newton, N. A., Gibson, F., Snoswell, A. M. & Hamilton, J. A. (1970) *Biochem. J.* **117**, 551–562
- Cox, G. B., Newton, N. A., Butlin, J. D. & Gibson, F. (1971) *Biochem. J.* **125**, 489–493
- Cox, G. B., Gibson, F. & McCann, L. (1973a) *Biochem. J.* **134**, 1015–1021
- Cox, G. B., Gibson, F., McCann, L. M., Butlin, J. D. & Crane, F. L. (1973b) *Biochem. J.* **132**, 689–695
- Cox, G. B., Gibson, F. & McCann, L. (1974) *Biochem. J.* **138**, 211–215
- Ernster, L. & Lee, C. (1967) *Methods Enzymol.* **10**, 738–744
- Haddock, B. A. & Downie, J. A. (1974) *Biochem. J.* **142**, 703–706
- Hayes, W. (1968) in *Experiments in Microbial Genetics* (Clowes, R. C. & Hayes, W., eds.), pp. 157–158, Blackwell Scientific Publications, Oxford
- King, E. J. (1932) *Biochem. J.* **26**, 292–297
- Low, K. B. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 160–167
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Luria, S. E. & Burrous, J. W. (1957) *J. Bacteriol.* **74**, 461–476
- Monod, J., Cohen-Bazire, G. & Cohn, M. (1951) *Biochim. Biophys. Acta* **7**, 585–599
- Novick, R. P., Clowes, R. C., Cohen, S. N., Curtiss, R., Datta, N. & Falkow, S. (1976) *Bacteriol. Rev.* **40**, 168–189
- Pittard, J. (1965) *J. Bacteriol.* **89**, 680–686
- Simoni, R. D. & Postma, P. W. (1975) *Annu. Rev. Biochem.* **44**, 523–554
- Taylor, A. L. & Thoman, M. S. (1964) *Genetics* **50**, 659–677