The *uncA* Gene Codes for the α -Subunit of the Adenosine Triphosphatase of *Escherichia coli*

ELECTROPHORETIC ANALYSIS OF *uncA* MUTANT STRAINS

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Four mutant strains of *Escherichia coli* which lack membrane-bound adenosine triphosphatase activity were shown by genetic-complementation tests to carry mutations in the *uncA* gene. A soluble inactive F_1 -ATPase aggregate was released from the membranes of three of the *uncA* mutant strains by low-ionic-strength washing, and purified by procedures developed for the purification of F_1 -ATPase from normal strains. Analysis of the subunit structure by two-dimensional gel electrophoresis indicated that the F_1 -ATPase in strains carrying the *uncA401* or *uncA453* alleles had a subunit structure indistinguishable from normal F_1 -ATPase. In contrast, the F_1 -ATPase from the strain carrying the *uncA447* allele contained an α -subunit of normal molecular weight, but abnormal net charge. Membranes from strains carrying the *uncA450* allele did not have F_1 -ATPase aggregates that could be solubilized by low-ionic-strength washing. However, a partial diploid strain carrying both the *uncA*⁺ and *uncA450* alleles formed an active F_1 -ATPase aggregate which could be solubilized by low-ionic-strength washing of the membranes and which contained two types of α -subunit, one of which was normal and the other had abnormal net charge. It is concluded that the *uncA* gene codes for the α -subunit of the adenosine triphosphatase.

Mutant strains of *Escherichia coli* K12 in which electron transport is uncoupled from phosphorylation have been grouped into those that have retained ATPase activity and those that have not (see Haddock & Jones, 1977). Genetic complementation has allowed the further subdivision of these groups, with mutant strains carrying mutations in the *uncB*, *uncC* or *uncE* genes retaining ATPase activity and those carrying mutations in the *uncA* or *uncD* genes having lost ATPase activity (Gibson *et al.*, 1977b; Cox *et al.*, 1978a; Downie *et al.*, 1979). These five genes have been shown to be components of an operon and are transcribed in the order *uncBEADC* (Gibson *et al.*, 1978; Downie *et al.*, 1979).

The *uncD* gene codes for the β -subunit of the F_1 portion of the ATPase. Mutations in the *uncD* gene have been shown to result in the formation of a

β -subunit of abnormal net charge and the properties of the membranes vary depending on the particular mutant β -subunit present (Senior *et al.*, 1979). Evidence has been obtained (Dunn, 1978; Kanazawa *et al.*, 1978) that the *uncA* gene codes for the α -subunit of the ATPase. Thus ATPase activity could be restored to the inactive F_1 -ATPase from a mutant strain carrying the *uncA401* allele by reconstitution of the dissociated complex with an excess of the normal α -subunit. The work described in the present paper confirms the conclusions of Dunn (1978) and Kanazawa *et al.* (1978) in that two of four strains carrying mutations in the *uncA* gene are found to form an α -subunit of abnormal net charge.

Materials and Methods

Chemicals

Poly(ethylene glycol) 6000 was obtained from BDH Chemicals, Poole, Dorset, U.K. All chemicals used were of the highest quality available.

Organisms

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

Abbreviations used: ATPase, Mg^{2+} -stimulated adenosine triphosphatase (EC 3.6.1.3); Tes, 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulphonic acid.

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

Bacterial strain	Relevant genetic loci	Other information
AN249	<i>uncA401, argH, entA</i>	Cox <i>et al.</i> (1973)
AN346	<i>ilvC, argH, pyrE, entA</i>	
AN732	<i>argH, pyrE, metE, entA</i>	
G31	<i>uncA447, argH, pyrE, metE, entA</i>	
AN1111	<i>uncA447, argH, pyrE, entA</i>	Isolated from strain AN732 after treatment with <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
G65	<i>uncA453, argH, pyrE, metE, entA</i>	Isolated after transduction with strain G31 as donor and strain AN346 as recipient
AN1137	<i>uncA453, argH, pyrE, entA</i>	Isolated from strain AN732 after treatment with <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
G88	<i>uncA450, argH, pyrE, metE, entA</i>	Isolated after transduction with strain G65 as donor and strain AN346 as recipient
AN1113	<i>uncA450, argH, pyrE, entA</i>	Isolated from strain AN732 after treatment with <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
AN862	F' (pAN11), <i>ilvC, argH, pyrE, purE, recA, nalA</i>	Isolated after transduction with strain G88 as donor and strain AN346 as recipient
AN1164	F' (pAN11), <i>uncA450, argH, pyrE, entA, recA, nalA</i>	Cox <i>et al.</i> (1978a); the plasmid pAN11 carried a normal <i>unc</i> operon
		Prepared by the method described by Gibson <i>et al.</i> (1977b)

Other methods

Media and growth of organisms, genetic techniques, preparation of cell membranes, washing of cell membranes in low-ionic-strength buffer, assay of ATPase activity, determination of protein, two-dimensional gel electrophoresis and measurement of atebirin-fluorescence quenching are described in Senior *et al.* (1979).

Purification of F_1 -ATPase

The methods of Vogel & Steinhart (1976), Bragg & Hou (1972) and Futai *et al.* (1974) were used in attempts to purify F_1 -ATPase from partial diploid strain AN862 (*unc⁺/unc⁺*) and from the normal strain AN248. None of these methods gave a pure preparation which was capable of reconstituting ATP-dependent atebirin-fluorescence quenching or ATP-dependent transhydrogenase activity in F_1 -ATPase-depleted membranes. This confirms the findings of others (Sternweis & Smith, 1977; Larson & Smith, 1977) that a given purification procedure may give varying results when applied to different *E. coli* strains, or to the same strain grown in different media. However, by combining features of these methods, a rapid method was devised, which can be used with equal confidence for the preparation of either normal F_1 -ATPase or inactive F_1 -ATPase from mutant strains. All steps were carried out at 4°C.

Step 1: solubilization of F_1 -ATPase. The F_1 -ATPase was solubilized by washing the membranes in low-ionic-strength buffer as described by Senior *et al.* (1979). Briefly, membranes were washed twice

in low-ionic-strength buffer containing 5 mM-Tes, 15% (v/v) glycerol, 0.5 mM-dithiothreitol, 40 mM-6-aminohexanoic acid and 6 mM-*p*-aminobenzamidine. As shown previously (Cox *et al.*, 1978b) about 70% of the protein is washed from the membranes by this procedure, but the ATPase activity remains membrane-bound due to the presence of *p*-aminobenzamidine. It had been concluded (Cox *et al.*, 1978b) that the effect of the *p*-aminobenzamidine was due to inhibition of a proteinase, but subsequent work has indicated that the retention of the F_1 -ATPase on the membrane is due to binding of the *p*-aminobenzamidine to the membrane-bound ATPase complex (J. A. Downie, unpublished observation). Subsequent washing twice with the same buffer system, but lacking *p*-aminobenzamidine, releases all the ATPase activity into the supernatant. The 6-aminohexanoic acid, another known proteinase inhibitor, is included in the buffer system in order to stabilize the F_1 -ATPase (see Cox *et al.*, 1978b).

Step 2: treatment with $MgCl_2$ followed by precipitation with poly(ethylene glycol). Tes buffer (pH 7.0) was added to the crude soluble F_1 -ATPase preparation from a 1 M stock solution to give a final concentration of 50 mM, dithiothreitol was added from a 0.5 M stock solution to 1 mM and $MgCl_2$ was added from a 1 M stock solution (pH 7.0) to 50 mM. The solution was centrifuged for 1 h at 8000g and the pellet discarded. Poly(ethylene glycol) (PEG 6000; 50%, w/v, in water) was added to give 10% (w/v) final concentration and the turbid solution was centrifuged for 1 h at 8000g. The white pellet was retained and dissolved in 'column buffer', which contained Tris/HCl (50 mM, pH 7.4), glycerol (10%, v/v), EDTA (2 mM), ATP (1 mM), dithiothreitol (1 mM) and

6-aminohexanoic acid (40mM); 50ml of column buffer was used to dissolve the protein obtained from 200g of cells. Just before use, phenylmethanesulphonyl fluoride was added from an ethanolic stock solution (40mM) to the column buffer to give 1mM final concentration. The solution was centrifuged for 15min at 50000g and the brown pellet discarded.

Step 3: ion-exchange chromatography on Whatman DE52 cellulose. The cellulose column (3.1 cm $\times$ 4.5 cm) was equilibrated with the column buffer described above. The enzyme solution was applied and 50ml of column buffer was used to wash through unadsorbed proteins; 100ml of column buffer fortified with 40mM-Na₂SO₄ (pH 7.4) was applied and the effluent discarded. Then 200ml of column buffer fortified with 80mM-Na₂SO₄ (pH 7.4) was passed through the column and the eluate concentrated to about 5ml using an Amicon Diaflo pressure filtration system and UM 10 membranes. The concentrate was centrifuged for 20min at 40000g and the pellet discarded.

Step 4: gel filtration. A Sepharose CL-6B column (2.5 cm $\times$ 95 cm) was equilibrated with column buffer fortified with 40 mM-Na₂SO₄ (pH 7.4), which was passed upwards through the column at a flow rate of 12–15 ml/h. Only one protein peak was obtained, eluting at about 270 ml. The peak fractions were

concentrated by ultrafiltration as above. The enzyme was stored frozen at -20°C and remained stable for at least 3 months.

Results

Characterization of uncA mutants by genetic complementation analysis

Mutant strains carrying the *uncA401* allele are unable to grow on succinate, have low aerobic growth yields, and lack membrane-bound ATPase activity (Butlin *et al.*, 1971). Three new mutant strains unable to grow on succinate (G31, G65 and G88) were isolated after mutagenesis of strain AN732 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and found to have a similar phenotype to strains carrying the *uncA401* allele. The three mutant alleles *unc-447*, *unc-453* and *unc-450* present in strains G31, G65 and G88 respectively, were transferred by cotransduction with the *ilv* genes into strain AN346 to give strains AN1111 (*unc-447*), AN1137 (*unc-453*) and AN1113 (*unc-450*). The *unc-447*, *unc-453* and *unc-450* alleles were then incorporated into F-plasmids using the method described previously (Gibson *et al.*, 1977b). The resulting partial diploid strains were then used as donor strains in complementation tests with female strains carrying the *uncA401*, *uncB402*,

Fig. 1. Atebrin-fluorescence quenching in membranes from various *uncA* mutant strains

Atebrin-fluorescence quenching was measured as described by Gibson *et al.* (1977a). Conditions for reconstitution were as described by Senior *et al.* (1979), with about 1 mg of membrane protein and about 200 μg of F₁-ATPase purified from strain AN862 (*unc⁺/unc⁺*). (a) Trace obtained with low-ionic-strength washed membranes from strain AN1111 (*uncA447*) prepared in the presence of *p*-aminobenzamidine. Similar results were obtained with membranes from strains AN249 (*uncA401*), AN1137 (*uncA453*) or AN1113 (*uncA450*). A similar trace was also obtained with membranes from strain AN1113 (*uncA450*) which had been subsequently washed in the absence of *p*-aminobenzamidine. (b) Trace obtained with membranes from strain AN1111 (*uncA447*) washed with low-ionic-strength buffer, but in the absence of *p*-aminobenzamidine. Similar results were obtained with membranes from strains AN249 (*uncA401*) or AN1137 (*uncA453*). (c) Trace obtained, after the reconstitution by normal F₁-ATPase, with membranes from strain AN1111 (*uncA447*) washed with low-ionic-strength buffer in the absence of *p*-aminobenzamidine. Similar reconstitution was obtained with membranes from strains AN249 (*uncA401*) or AN1137 (*uncA453*). No reconstitution of ATP-dependent atebrin-fluorescence quenching was obtained after addition of normal F₁-ATPase to low-ionic-strength washed membranes from strain AN1113 (*uncA450*), and the trace was essentially the same as that shown in (a).

Table 2. Purification of F_1 -ATPase from strain AN862 (*unc⁺/unc⁺*)

Details of the method of purification are given in the Materials and Methods section; 200 g of cells was used as starting material.

		Protein (mg)	ATPase (μ mol/min per mg of protein)
Step 1	Pooled low-ionic-strength washes	1265	1.1
Step 2	Poly(ethylene glycol) precipitate	245	4.1
Step 3	Concentrated 80mM- Na_2SO_4 eluate from the Whatman DE52 column	39	7.8
Step 4	Sephacose CL-6B column; pooled active fractions after concentration	27	10

Fig. 2. Gel filtration on a Sepharose CL-6B column of F_1 -ATPase prepared from membranes of strain AN862 (*unc⁺/unc⁺*) or strain AN1111 (*uncA447*).

Details of the procedure for purifying F_1 -ATPase are given in the Materials and Methods section, and the gel filtration, the elution profile of which is shown, is the final step. (a) Strain AN862 (*unc⁺/unc⁺*); (b) strain AN1111 (*uncA447*). The column was 2.5 $\times$ 95 cm and the elution volume of the protein peak was about 270 ml. ●, Protein expressed as A_{650} by the method of Lowry *et al.* (1951); ○, ATPase activity expressed as A_{660} by the method of Senior & Brooks (1970).

uncC424, *uncD409* or *uncE429* alleles as well as with female strains carrying the *unc-447*, *unc-453* or *unc-450* alleles. If genetic complementation occurs, colonies are formed on the succinate medium in the region where the donor and recipient strains mix in the cross-streaking test used. Complementation was observed between the *unc-447*, *unc-453* or *unc-450* alleles and the *uncB402*, *uncC424*, *uncD409* or *uncE429* alleles. Complementation was not observed between the new *unc* mutant alleles and the *uncA401* allele, nor was complementation observed between the *unc-447*, *unc-453* and *unc-450* alleles themselves. It was concluded that the *unc-447*, *unc-453* and *unc-450* alleles affect the *uncA* gene and were accordingly designated *uncA447*, *uncA453* and *uncA450*.

Reconstitution of ATP-dependent atebtrin-fluorescence quenching

Membrane preparations from the normal strain AN248 retained ATPase activity and both NADH- and ATP-dependent atebtrin-fluorescence quenching

after washing in low-ionic-strength buffer in the presence of *p*-aminobenzamidine (Cox *et al.*, 1978b). If these membranes were subsequently washed in low-ionic-strength buffer in the absence of *p*-aminobenzamidine then the membranes lost all three activities due to the removal of the F_1 -ATPase (Cox *et al.*, 1978b).

Membrane preparations from strains AN249 (*uncA401*), AN1111 (*uncA447*), AN1137 (*uncA453*) and AN1113 (*uncA450*), were washed in low-ionic-strength buffer in the presence of *p*-aminobenzamidine. The washed membranes did not have ATP-induced atebtrin-fluorescence-quenching activity, but retained the NADH-induced atebtrin-fluorescence-quenching activity (Fig. 1). The addition of purified F_1 -ATPase from strain AN862 (*unc⁺/unc⁺*) did not reconstitute the ATP-dependent activity. If the membranes from strains AN249 (*uncA401*), AN1111 (*uncA447*) or AN1137 (*uncA453*) were subsequently washed in low-ionic-strength buffer in the absence of *p*-aminobenzamidine, then both the NADH- and ATP-dependent atebtrin-fluorescence-quenching activities were absent; the addition of purified F_1 -ATPase from strain AN862 (*unc⁺/unc⁺*) reconstituted both activities (Fig. 1). However, if the membranes from strain AN1113 (*uncA450*) were also subsequently washed in the absence of *p*-aminobenzamidine they retained NADH-induced atebtrin-fluorescence-quenching activity. Furthermore, the ATP-dependent activity could not be reconstituted by the addition of purified F_1 -ATPase from strain AN862 (*unc⁺/unc⁺*) (Fig. 1) (see below).

Purification of normal F_1 -ATPase and F_1 -ATPase from *unc* mutant strains

A summary of the yields and activities obtained when F_1 -ATPase was prepared from the partial diploid strain AN862 (*unc⁺/unc⁺*) is shown in Table 2. The specific activity of the ATPase is low compared with other published values (see Bragg & Hou, 1972; Vogel & Steinhart, 1976; Futai *et al.*, 1974). However, with respect to the reconstitution of energy-linked reactions the purified enzyme is as active, in terms of mg of protein required per mg of washed

(a) *uncA453* (b) *uncA453* + *uncA447* (c) *uncA447*

Fig. 3. Two-dimensional gel electrophoresis of purified F₁-ATPase from strains AN1137 (*uncA453*) and AN1111 (*uncA447*)

Purified F₁-ATPase was electrophoresed as described by Senior *et al.* (1979) over a pH range of about 7.5–4.5. (a) Purified F₁-ATPase (about 15 μ g) from strain AN1137 (*uncA453*). The α -, β -, γ -, δ - and ϵ -subunits are marked and each of the subunits is indistinguishable from normal by co-electrophoresis. (b) Mixture of purified F₁-ATPase preparations (about 15 μ g of each) from strains AN1137 (*uncA453*) and AN1111 (*uncA447*). (c) Purified F₁-ATPase (about 15 μ g) from strain AN1111 (*uncA447*). Only the sections of the gels covering the pH range 7.5–5.0 in the first dimension are shown. In (a) the complete molecular weight range is shown, but in (b) and (c) only those regions of the gels containing peptides of molecular weights greater than 25000 are shown.

membranes, as any of the preparations referred to above (J. A. Downie, unpublished results). About 50 μ g of purified F₁-ATPase gave 50% saturation of binding sites on 1 mg of F₁-ATPase-depleted normal membranes of the haploid strain AN248 (Downie *et al.*, 1979). Two-dimensional gel electrophoresis showed five polypeptides corresponding to the α -, β -, γ -, δ - and ϵ -subunits of F₁-ATPase (see Fig. 3).

It was apparent from the atebrin-fluorescence-quenching data (see above) that strains AN1111 (*uncA447*) and AN1137 (*uncA453*) were similar to AN249 (*uncA401*) and that an inactive F₁-ATPase might be removed from the membranes of these strains by low-ionic-strength washing in the absence of *p*-aminobenzamidine. The method used for the solubilization and purification of normal F₁-ATPase was therefore used with membrane preparations from each of these three *uncA* mutant strains. A typical elution profile for the final step of the purification procedure (Sephacrose CL-6B column) is shown in Fig. 2. The F₁-ATPase from each of the mutant strains had no detectable ATPase activity.

Analysis of purified F₁-ATPase from the uncA mutant strains by two-dimensional gel electrophoresis

Purified F₁-ATPase isolated from each of strains AN249 (*uncA401*), AN1111 (*uncA447*) and AN1137 (*uncA453*) was dissociated by heating at 100°C in the presence of sodium dodecyl sulphate and mercaptoethanol (Cox *et al.*, 1978b) with protein concentrations of about 2.5 mg/ml. These samples were then analysed by two-dimensional gel electrophoresis. The β -, γ -, δ - and ϵ -subunits of the inactive ATPases from each of these *uncA* mutant strains had the same molecular weights and isoelectric points as the corresponding subunits from a normal F₁-ATPase (see Fig. 3). However, the α -subunit of the F₁-ATPase from strain AN1111 (*uncA447*) had the same molecular weight but a different isoelectric point to the α -subunit from a normal F₁-ATPase or from the other *uncA* mutants AN249 (*uncA401*) or AN1137 (*uncA453*) (Fig. 3). The α -subunits in strains AN249 (*uncA401*) and AN1137 (*uncA453*) are presumably abnormal, but the alteration does not result in a molecular weight, or charge, difference that can be detected by the methods used.

The apparent isoelectric point of the mutant *uncA453* ' α '-subunit is about 6.4, the same as the normal α -subunit, whereas the mutant *uncA447* ' α '-subunit has an apparent isoelectric point of about 6.3. The unlabelled arrow indicates a polypeptide which is found in purified F₁-ATPase preparations and which is used for reference.

Fig. 4. Two-dimensional gel electrophoresis of F_1 -ATPase purified from the partial diploid strain AN1164 ($unc^+/uncA450$)

Purified F_1 -ATPase (15 μ g of protein) from strain AN1164 ($unc^+/uncA450$) was electrophoresed as described by Senior *et al.* (1979) over a pH range of about 7.5–4.5. The normal α -, β -, γ -, δ - and ϵ -subunits and the abnormal ' α' '-subunit are marked on the electrophoretogram. The abnormal ' α' '-subunit has an apparent isoelectric point of about 6.5. The unlabelled arrow indicates a polypeptide which is found in purified F_1 -ATPase preparations and which is used for reference.

Identification of an abnormal α -subunit in the purified F_1 -ATPase from the partial diploid strain AN1164 ($unc^+/uncA450$)

The addition of purified F_1 -ATPase from a normal strain to washed membranes from strain AN1113 ($uncA450$) does not result in the reconstitution of ATP-dependent atebirin-fluorescence quenching (see above). In this respect the washed membranes are similar to those from mutant strains carrying the *uncD409* allele in which an abnormal β -subunit was tightly bound to the membrane (Fayle *et al.*, 1978). However, this subunit could be washed off the membranes from a partial diploid strain carrying both the *uncD409* allele and a normal *uncD* allele (Fayle *et al.*, 1978). Membranes from the partial diploid strain AN1164 ($unc^+/uncA450$) were therefore prepared and an F_1 -ATPase aggregate was solubilized and purified by the method used for the normal F_1 -ATPase. The purified F_1 -ATPase from strain AN1164 ($unc^+/uncA450$) had a specific activity of 8.5. The F_1 -ATPase was dissociated as described above and the subunit structure analysed by two-dimensional gel electrophoresis. The β -, γ -, δ - and ϵ -subunits of the F_1 -ATPase had the same molecular weights and isoelectric points as the corresponding subunits from a normal F_1 -ATPase (Fig. 4). However, there were two polypeptides with the same molecular weight as the α -subunit, but with different isoelectric points. The mutant α -subunit had a higher isoelectric point than the normal α -subunit (Fig. 4).

Discussion

The presence of α -subunits with abnormal isoelectric points in strains AN1111 (*uncA447*) and AN1113 (*uncA450*) confirm that the *uncA* gene codes for the α -subunit of the ATPase (Dunn, 1978; Kanazawa *et al.*, 1978). It would appear from the results with strains AN249 (*uncA401*) and AN1137 (*uncA453*) that mutations affecting the α -subunit leading to loss of ATPase activity do not necessarily result in an alteration of charge. This is in contrast with the effect of mutations in the *uncD* gene where it is notable that all five mutant strains so far examined form β -subunits with altered isoelectric points (Senior *et al.*, 1979).

The properties of the membranes from strain AN1113 (*uncA450*) are similar to the properties of some *uncD* mutants in which abnormal β -subunits of the ATPase are attached to the membrane and cannot be removed by low-ionic-strength washing (Fayle *et al.*, 1978; Senior *et al.*, 1979). Such results emphasize the difficulty of determining, on the basis of biochemical studies, whether a particular mutation affects the F_0 or the F_1 portions of the ATPase (see Gibson *et al.*, 1977b; Rosen *et al.*, 1978). Thus, for example, the lack of reconstitution of ATP-dependent atebirin-fluorescence quenching by the addition of purified normal F_1 -ATPase to washed membranes from strain AN1113 (*uncA450*) might lead to the incorrect conclusion that this particular mutation affected a component of the F_0 portion of the ATPase.

Analysis of membranes from strain AN1113 (*uncA450*), by two-dimensional gel electrophoresis, indicated that both the abnormal α -subunit and the normal β -subunit were retained on the membrane and could not be removed by low-ionic-strength washing (G. B. Cox, unpublished observations). It would appear therefore that the presence of this abnormal α -subunit prevents the loss of normal β -subunits from the membrane during low-ionic-strength washing, but abnormal β -subunits, as found in most *uncD* mutants, do not prevent the loss from the membrane of normal α -subunits (Senior *et al.*, 1979).

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References

- Bragg, P. D. & Hou, C. (1972) *FEBS Lett.* **28**, 309–312
- Butlin, J. D., Cox, G. B. & Gibson, F. (1971) *Biochem. J.* **124**, 75–81
- Cox, G. B., Gibson, F. & McCann, L. (1973) *Biochem. J.* **134**, 1015–1021

- Cox, G. B., Downie, J. A., Gibson, F. & Radik, J. (1978a) *Biochem. J.* **170**, 593–598
- Cox, G. B., Downie, J. A., Fayle, D. R. H., Gibson, F. & Radik, J. (1978b) *J. Bacteriol.* **133**, 287–292
- Downie, J. A., Senior, A. E., Gibson, F. & Cox, G. B. (1979) *J. Bacteriol.* in the press
- Dunn, S. D. (1978) *Biochem. Biophys. Res. Commun.* **82**, 596–602
- Fayle, D. R. H., Downie, J. A., Cox, G. B., Gibson, F. & Radik, J. (1978) *Biochem. J.* **172**, 523–531
- Futai, M., Sternweis, P. C. & Heppel, L. A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2725–2729
- Gibson, F., Cox, G. B., Downie, J. A. & Radik, J. (1977a) *Biochem. J.* **162**, 665–670
- Gibson, F., Cox, G. B., Downie, J. A. & Radik, J. (1977b) *Biochem. J.* **164**, 193–198
- Gibson, F., Downie, J. A., Cox, G. B. & Radik, J. (1978) *J. Bacteriol.* **134**, 728–736
- Haddock, B. A. & Jones, C. W. (1977) *Bacteriol. Rev.* **41**, 47–99
- Kanazawa, H., Saito, S. & Futai, M. (1978) *J. Biochem. (Tokyo)* **84**, 1513–1517
- Larson, R. J. & Smith, J. B. (1977) *Biochemistry* **16**, 4266–4270
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Rosen, B. P., Brey, R. N. & Hasan, S. M. (1978) *J. Bacteriol.* **134**, 1030–1038
- Senior, A. E. & Brooks, J. C. (1970) *Arch. Biochem. Biophys.* **140**, 257–266
- Senior, A. E., Fayle, D. R. H., Downie, J. A., Gibson, F. & Cox, G. B. (1979) *Biochem. J.* **180**, 111–118
- Sternweis, P. C. & Smith, J. B. (1977) *Biochemistry* **16**, 4020–4025
- Vogel, G. & Steinhart, R. (1976) *Biochemistry* **15**, 208–216