The biochemical and genetic approach to the study of bioenergetics with the use of *Escherichia coli*: progress and prospects

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How the ‘energy currency’ of the cell, adenosine triphosphate (ATP), is produced consequent upon the oxidation of foodstuffs (oxidative phosphorylation) is, despite prolonged research, still a matter of debate and the molecular mechanism of the process is unknown. It appears that the problem of oxidative phosphorylation can be approached with the aid of the biochemical genetics of the bacterium *Escherichia coli*. The ease of manipulation of bacteria and definitive results obtained by this approach have been invaluable in solving other major biochemical problems. Mutants affected in oxidative phosphorylation have been isolated and characterized by genetic and biochemical techniques. These ‘uac’ mutants are affected in the adenosine triphosphatase (ATPase) multiprotein complex which is part of the cell membrane and responsible for the terminal stages of ATP synthesis. Seven distinct genes concerned with oxidative phosphorylation have been characterized in *E. coli* and shown to be part of an operon. The relationships between the different classes of *uac* genes and the various components of the ATPase have been established. Information about the assembly of the ATP synthesizing complex in the cell membrane has also been obtained and the stage set for further studies on the assembly, control and function of the ATP synthesizing system.

**Introduction**

I am conscious of the honour of being invited to deliver a Leeuwenhoek Lecture, which is perhaps of special significance to me as I have long been an admirer of Antony van Leeuwenhoek, his work and his approach to experimental science. My introduction to van Leeuwenhoek was through the remarkable book written by a Fellow of the Royal Society, Clifford Dobell (1932), and my interest was reinforced by C. B. van Neil during his lectures at Pacific Grove. At least in some parts of the Antipodes, van Leeuwenhoek’s work is well recognized. When additions were made to the old Bacteriology School in the University of Melbourne in about 1940, the names of Koch, Pasteur and Lister were displayed prominently on the front of the building. However, in the new Microbiology School, which was occupied in 1965, the model of an intended sculpture representing a van Leeuwenhoek
microscope (figure 1) occupies pride of place in the foyer. Unfortunately the final sculpture, which was planned to be about 10 feet (ca. 3 m) high and on the exterior of the building, never materialized.

This lecture concerns the key compound in the energetics of living cells, namely adenosine triphosphate (ATP). It is 50 years since Engelhardt (1930, 1932) showed that ATP played an important role in aerobic respiration, and these observations were followed several years later by the development of the concept of oxidative phosphorylation in which substrates are oxidized and, during the flow of electrons...
Figure 2. Diagram of electron transport chain (Downie & Cox 1978) and subunits of $F_1$-$F_0$ ATPase in *E. coli*. 
from substrate to oxygen via the carriers of the electron transport chain, ATP is generated from adenosine diphosphate (ADP) (figure 2) (see Slater 1981).

The problem of how energy is obtained from foodstuffs is one of the oldest problems in biochemistry and one of the most refractory. It is still not understood, at the molecular level, how ATP is formed as a result of oxidative phosphorylation although the transfer of protons across or within membranes appears to play an important role in the process (see Mitchell 1977; Williams 1978). The final step in the synthesis of ATP in mitochondria and chloroplasts has been recognized for many years as being a function of a multiprotein membrane-bound complex, adenosine triphosphatase (ATPase), capable of both synthesizing ATP from ADP and carrying out the reverse reaction to drive energy-requiring processes.

Over the last 30 years bacterial mutants, and particularly those of *Escherichia coli* K12 which is amenable to genetic manipulation, have played a central role in understanding various cellular processes such as DNA and protein synthesis. It is not clear why, considering the spectacular successes following studies on the biochemical genetics of *E. coli* in the 1950s and 1960s, a much earlier start was not made on the problem of oxidative phosphorylation with use of this organism. Possibly the preoccupation of workers on oxidative phosphorylation with mitochondria and chloroplasts, and the apparent low efficiency of oxidative phosphorylation in *E. coli*, led them to neglect this organism. Indeed it was not until the 1970s that it was generally realized that the ATPase of *E. coli* had many features in common, both structurally and functionally, with the ATPase of chloroplasts and mitochondria.

The membrane-bound ATPase is most conveniently discussed in terms of two distinct components F₁ and F₀. The F₁ ATPase is a protein complex that can be dissociated from the membrane. It consists of five different subunits in *E. coli* in the proportions α₃β₃γδε, and is able to hydrolyse ATP to ADP. The F₀ ATPase is a protein complex that appears to form a proton channel and consists in *E. coli* of three proteins of relative molecular masses about 24000, 18000 and 8400 (figure 2). The structure, in general terms, is the same for mitochondrial and chloroplast ATPases although the number of subunits, particularly in the F₀ portion, is not yet clearly defined. The complete F₁–F₀ ATPase complex is required for oxidative phosphorylation.

Dr Graeme B. Cox and I have been concerned, along with the other members of our group, for over a decade, in a study of the biochemical genetics of the ATPase of *E. coli*. In this lecture I wish to discuss, with particular reference to the work in our laboratory, the progress made and to indicate the current lines of work, and the prospects for the future.

**Isolation and Preliminary Characterization of Mutants**

Our entry into the field of oxidative phosphorylation was perhaps unusual. We actually had the appropriate mutants in our stock cultures for some time before starting on the problem. We were, for many years, interested in the biosynthesis of aromatic compounds and this work eventually led to a search for mutants unable to form ubiquinone, a component of the electron transport chain. An indirect
method of selection had to be devised that used the fact that \textit{E. coli} is a facultative anaerobe, able to obtain energy either by fermentation or via the electron transport chain. Mutants were therefore sought that were able to grow on glucose but unable to grow on a non-fermentable substrate such as succinate as sole source of carbon. For the oxidation of the latter compound the electron transport chain had to be involved. Many such strains were isolated and about 5–10\% of these were found to lack ubiquinone (Cox \textit{et al.} 1968; Young \textit{et al.} 1973).

During the study of ubiquinone biosynthesis it was realized that \textit{ubi}~\textsuperscript{–} mutants would provide excellent tools for studying ubiquinone function. While this work (Cox \textit{et al.} 1970) was in progress it was further realized that, among the strains unable to grow on succinate, there would be not only mutants affected in the electron transport chain itself, but also mutants affected in oxidative phosphorylation. The \textit{Ubi+} strains unable to grow on succinate were then screened to find those in which the electron transport chain was normal and the growth yield low. The latter characteristic could be simply determined by growing the mutant strains on limiting concentrations of glucose and measuring the final turbidity of the cultures. It was to be expected that strains that were affected in oxidative phosphorylation ('uncoupled' strains) would be growing at the expense of ATP generated by glycolysis. This gives a much lower yield of ATP than that given by oxidative phosphorylation and therefore the amount of cell material formed by uncoupled strains would be much less than that formed by normal strains. Strains with the expected phenotype were found (table 1) and the first examined in detail showed that it not only had lost the ability to carry out oxidative phosphorylation (table 1) but also lacked membrane-bound ATPase activity. This result provided an \textit{in vivo} confirmation of the work with mitochondria which indicated that ATPase is a 'coupling factor' in oxidative phosphorylation (Penefsky \textit{et al.} 1960). The gene affected in this mutant strain was designated \textit{uncA} (Butlin \textit{et al.} 1971). Other uncoupled strains were isolated in which the level of ATPase activity was normal (table 1) (Gutnick \textit{et al.} 1972; Butlin \textit{et al.} 1973).

Genetic mapping of the mutants now isolated in a number of laboratories shows that all of the genes affected in the various uncoupled strains map in the same region of the \textit{E. coli} genome, close to the \textit{ilv} gene cluster (see Downie \textit{et al.} 1979). Therefore it was not practicable to distinguish mutations in different \textit{unc} genes by simple genetic mapping.

It was previously known that the \textit{F}~\textsubscript{1} ATPase could be stripped from some bacterial membranes by washing them with low ionic strength buffer (see Downie \textit{et al.} 1979). Thus it was possible to carry out reconstitution experiments by washing the membranes and mixing the solutions of \textit{F}~\textsubscript{1} from various mutant strains with the stripped membranes from other mutant strains in the presence of Mg\textsuperscript{2+} and testing for the ability of mixtures of various fractions to carry out oxidative phosphorylation. The simpler test of ATP-dependent atein fluorescence quenching which is thought to monitor membrane energization (Kraayenhof 1970; Lee 1974) has proved most useful in facilitating experiments on membrane reconstitution.

By such reconstitution experiments it was shown that in the \textit{uncA}~\textsuperscript{–} strain, the defect was in the \textit{F}~\textsubscript{1} ATPase while in the ATPase~\textsuperscript{+} strain the defect was in the \textit{F}~\textsubscript{0}
ATPase (Cox et al. 1973). These results indicated that different genes were affected in the two mutant strains and therefore the gene affected in the ATPase\(^+\) strain was designated \textit{uncB}.

**Genetic classification of mutants**

The phenotypic characteristics of all uncoupled mutants are much the same, apart from the presence or absence of ATPase activity (see Downie et al. 1979).

**Table 1. Some properties of normal and uncoupled strains of \textit{E. coli}**

<table>
<thead>
<tr>
<th>Strain</th>
<th>NADH oxidase activity</th>
<th>Aerobic growth yield</th>
<th>ATPase activity</th>
<th>P/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN180 (unc(^+))</td>
<td>525</td>
<td>170</td>
<td>160</td>
<td>0.18</td>
</tr>
<tr>
<td>AN120 (uncA401)</td>
<td>510</td>
<td>93</td>
<td>&lt;5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AN283 (uncB402)</td>
<td>475</td>
<td>95</td>
<td>180</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

\(\dagger\) NADH oxidase activities are expressed as nanomoles of oxygen consumed per minute per milligram of membrane protein.

\(\ddagger\) Aerobic growth yields were measured as the final turbidity in Klett units (10\(^6\) cells per millilitre gives a turbidity of ca. 40 Klett units) after growth in shaken flasks on a limiting concentration (4 \(\mu\text{M}\)) of glucose.

\(\S\) ATPase activities are expressed as nanomoles of inorganic phosphate released per minute per milligram of membrane protein.

\(\|\) The P/O ratio is the number of moles of inorganic phosphate esterified per mole of oxygen consumed during the oxidation of NADH.

Therefore it was necessary to devise a system relating new \textit{unc} mutants to the reference \textit{uncA} and \textit{uncB} mutant strains to decide whether the mutations in the other mutants had affected genes other than \textit{uncA} or \textit{uncB}. It seemed that a genetic complementation system would prove most useful. For such experiments mutant \textit{unc} alleles, along with adjacent \textit{unc} genes, had to be transferred to a plasmid which, in turn, would be used to construct partial diploid strains in which both the reference mutant allele and the mutant allele being investigated were present in the same cell. If the two mutations affected the same gene, the resulting partial diploid strain would still be uncoupled; if the mutations affected different genes then there would be a normal copy of each of the affected genes present in the cell, and the partial diploid strain might be expected to be normal.

After a number of abortive attempts to develop such a complementation system, it was found that a plasmid that normally carried the \textit{unc} and \textit{ilv} genes had suffered a deletion and both of these groups of genes had been lost. This ‘deleted’ plasmid provided a simple means by which the \textit{unc} genes could be inserted onto a plasmid for genetic complementation tests (Gibson et al. 1977). Such tests are very simple (figure 3) and rely on the fact that partial diploids with a complement of normal genes will grow on succinate By examining various \textit{unc} mutants, it has now been possible to distinguish, by genetic complementation tests, seven distinct \textit{unc} genes designated \textit{uncA}, \textit{B}, \textit{C}, \textit{D}, \textit{E}, \textit{F} and \textit{G}.
The observation that all the unc genes mapped in the same region of the *E. coli* chromosome made it at least possible that they formed an operon, i.e. that the DNA coding for all the genes was read as a single transcriptional unit. To test whether this was so, mutants were isolated in which the bacteriophage Mu was inserted in various unc genes. Such insertions cause polarity effects in operons and these can be detected by genetic complementation tests. Thus Mu inserted in an early gene in an operon prevents reading of all the subsequent genes. By such experiments (table 2) it was shown that the unc genes did form an operon (see Downie *et al.* 1979) and various classes of Mu-induced mutants allowed the gene order uncB(EF)A(DG)C to be postulated. The Mu-induced mutants available did not allow the ordering of the uncF and E or the uncG and D genes. The position of the uncG gene was determined during cloning of the unc genes (see below) and the order proved to be uncAGD. The inference of a gene order uncBFE, drawn from experiments with deleted plasmids (Downie *et al.* 1981), proved to be incorrect when it was shown unequivocally, by DNA sequencing, that the order for the genes coding for the F\textsubscript{0} ATPase subunits is uncBEF (Gay & Walker 1981b; L. Langman & D. Jans, unpublished).

It was shown, mainly by membrane reconstitution experiments such as those

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**Figure 3.** Genetic complementation tests between a male strain carrying an uncC mutant allele on a plasmid and female strains carrying uncA, uncB, uncC or uncD mutant alleles. The medium contained succinate as sole source of carbon. A culture of the male strain was streaked across the agar and allowed to dry, after which the female strain was cross streaked as indicated. Complementation is shown by growth where the cells from two strains have mixed. Some mutant alleles give only very sparse growth on complementation.
described earlier, that the \textit{unc}B, \textit{E} and \textit{F} genes coded for the \textit{F}_0 ATPase and that the \textit{unc}A, \textit{G}, \textit{D} and \textit{C} genes coded for the \textit{F}_1 ATPase. This gene order would seem consistent with the idea that \textit{F}_0 genes are transcribed first, the products assembled in the membrane to give a complete \textit{F}_0 and then the \textit{F}_1 components assembled to form the complete \textit{F}_1–\textit{F}_0 ATPase. As we will see later, this simple model does not represent the actual sequence of events.

\textbf{Table 2. Genetic complementation tests between \textit{Mu}-induced polar \textit{unc} mutants and strains carrying reference \textit{unc} alleles}

(Complementation tested by growth on succinate medium and various energy-linked reactions; Gibson \textit{et al.} 1978).  

\begin{tabular}{|l|l|}
\hline
\textit{Mu::unc} allele & class \\
\hline
\textit{unc}-413 & \textit{B}^\text{-}\textit{E}^\text{-}\textit{F}^\text{-}\textit{A}^\text{-}\textit{G}^-\textit{D}^-\textit{C}^- \\
\textit{unc}-414 & \textit{B}^\text{+}\textit{E}^\text{+}\textit{F}^\text{+}\textit{A}^\text{+}\textit{G}^+\textit{D}^+\textit{C}^+ \\
\textit{unc}-418 & \textit{B}^\text{+}\textit{E}^\text{+}\textit{F}^\text{+}\textit{A}^\text{+}\textit{G}^-\textit{D}^-\textit{C}^- \\
\textit{unc}-421 & \textit{B}^\text{+}\textit{E}^\text{+}\textit{F}^\text{+}\textit{A}^\text{+}\textit{G}^+\textit{D}^-\textit{C}^- \\
\textit{unc}-415 & \textit{B}^\text{+}\textit{E}^\text{+}\textit{F}^\text{+}\textit{A}^\text{+}\textit{G}^+\textit{D}^+\textit{C}^+ \\
\hline
\end{tabular}

Conclusion: The \textit{unc} genes form an operon read from the \textit{unc}B gene and the order is \textit{unc}B(\textit{EF})A(\textit{DG})C.

\textbf{Gene–polypeptide relationships}

\textbf{(a) \textit{F}_1 ATPase}

To decide how the different \textit{unc} genes related to the various known polypeptides in the \textit{F}_1 ATPase, a variety of methods have been used. The most straightforward method was to look for altered proteins in the membranes of the mutants. Abnormal proteins have been found in membranes from strains carrying mutant \textit{unc}A or \textit{unc}D alleles. Thus some \textit{unc}A mutants formed inactive \textit{F}_1 ATPase which could be purified following low ionic strength washing of the membranes. Two-dimensional gel electrophoresis showed that some mutants contained electrophoretically altered \textit{\alpha}-subunits, although the \textit{\alpha}-subunits in other mutants had a normal net charge (Senior \textit{et al.} 1979\textit{a}). That the \textit{unc}A gene codes for the \textit{\alpha}-subunit was also shown by Kanazawa \textit{et al.} (1978) and Dunn (1978), who reconstituted the ATPase activity in \textit{F}_1 from an \textit{unc}A mutant strain by the addition of purified normal \textit{\alpha}-subunits.

Most \textit{unc}D mutant strains did not form a normal \textit{F}_1 ATPase aggregate on the membranes, but in these mutants it was shown that an electrophoretically altered \textit{\beta}-subunit was firmly attached to the membranes (figure 4) (Fayle \textit{et al.} 1978; Senior \textit{et al.} 1979\textit{b}).

The gene–polypeptide relationships for the remaining known genes coding for the \textit{F}_1 ATPase were determined following cloning of the genes. Thus by using the restriction endonuclease \textit{HindIII} the \textit{unc} genes were cloned (Downie \textit{et al.} 1980) in two plasmids pAN51 and pAN36 (figure 5). These were found by genetic complementation tests to carry the genes for \textit{unc}BE\textit{FA} and \textit{unc}DC respectively (figure 5). When these two plasmids were used as source of DNA to reconstruct the whole operon in the plasmid pAN45 (figure 5) it was found that this plasmid,
**Figure 4.** Two-dimensional gel electrophoresis of membranes from (a), an unc\(^+\) strain and (b) and (c), two unc\(D\) mutants showing the abnormal \(\beta\)-subunits. Only the section of the gel around the position of the \(\beta\)-subunit is shown. The membranes were washed in low ionic strength buffer and the positions of polypeptides a, b, c, d and e serve as reference points (Senior et al. 1979b).

**Figure 5.** Maps of plasmids pAN51, pAN36 and pAN45 showing the approximate positions of the unc genes. The heavy lines indicate the vector (pACYC184) portion of the plasmid (Downie et al. 1980).
F. Gibson

Unlike either pAN51 or pAN36, carried a functional uncG gene indicating that this gene lay between the uncA and uncD genes (figure 5) and was inactivated by cutting with HindIII (Downie et al. 1980). *In vitro* protein synthesis experiments with the DNA of plasmid pAN45 in a coupled transcription–translation system in the presence of [35S]methionine showed that all the known subunits of the F1 ATPase were formed, together with several other polypeptides (figure 6) most of which were related to the DNA of the plasmid vector. Related observations were that, while the γ-subunit was formed from the plasmid pAN45 which carried the whole unc operon, this subunit was not formed when the DNA from either plasmid pAN51 or pAN36 was used alone for *in vitro* protein synthesis. These observations indicated that the uncG gene codes for the γ-subunit (Downie et al. 1980).

The result of *in vitro* protein synthesis experiments with DNA of plasmid pAN51 showed that the gene coding for the δ-subunit is carried on this plasmid (Downie et al. 1980). However, no unc mutants shown to have a defect in this subunit have yet been described. The gene for the δ-subunit lies between the uncF and uncA genes (Downie et al. 1981; Gay & Walker 1981 b; Mabuchi et al. 1981).

*In vitro* protein synthesis experiments with the plasmid pAN36 (uncDC) as the source of DNA showed that the β- and ε-subunits were formed, suggesting that the ε-subunit was coded for by the uncC gene. This result is consistent with previous observations (unpublished) that, in the cytoplasm of uncC mutants, an ATPase is found that differs from the soluble ATPase found in some other unc mutants in that it lacks the ε-subunit.
The 8400 relative molecular mass ($M_r$ 8400) $F_0$ ATPase subunit from both *E. coli* and other organisms has been extensively studied. It is an unusual protein, soluble in chloroform–methanol (Cattell *et al.* 1971) and the amino acid sequence for this protein from a number of sources has been ascertained (Sebald *et al.* 1979). This subunit binds the ATPase inhibitor dicyclohexylcarbodiimide (DCCD) and in both DCCD-resistant mutants and in uncoupled mutants the $M_r$ 8400 subunit has been examined and found to have an abnormal amino acid composition (Hoppe *et al.* 1980; Wachter *et al.* 1980). The membranes from some *uncE* mutant strains were shown to lack the $M_r$ 8400 protein (Downie *et al.* 1981) and other *uncE* mutants form altered $M_r$ 8400 proteins. For example, in the $M_r$ 8400 polypeptide from the strain carrying the *uncE410* allele the proline at position 64 in the peptide was found to be replaced by leucine (G. B. Cox, unpublished results). The *uncE* gene therefore codes for the $M_r$ 8400 subunit.

Comparison of two-dimensional gels of membrane proteins with corresponding radioautographs of $^{35}$S-labelled proteins synthesized in vitro allowed the location of the $M_r$ 18000 polypeptide of the $F_0$ ATPase to be determined on the two-dimensional gels. The examination of membranes from various mutants by two-dimensional gel electrophoresis showed that the $M_r$ 18000 polypeptide was absent from the membranes of the *uncF* mutants (Downie *et al.* 1981), and it is assumed that the *uncF* gene codes for this subunit.

As the *uncE* gene codes for the $M_r$ 8400 polypeptide and the *uncF* gene codes for the $M_r$ 18000 subunit it seemed likely that the *uncB* gene coded for the $M_r$ 24000 polypeptide. However, the $M_r$ 24000 subunit, like the $M_r$ 8400 subunit, cannot be seen in the two-dimensional gel system used (unpublished; R. Fillingame, personal communication) and the relationship between the *uncB* gene and the $M_r$ 24000 subunit was established with use of in vitro protein synthesis and plasmids. Thus a plasmid (pAN95) that carried a small deletion (about 100 base pairs) that inactivated the *uncB* gene was derived from the plasmid pAN51. The assumption was made that the $F_0$ ATPase components formed during an in vitro protein synthesis experiment would be incorporated into the membranes present in the incubation mixture used. At the conclusion of the protein synthesis, the membranes were removed by centrifugation, solubilized and examined by one-dimensional gel electrophoresis and radioautography. Whereas the $M_r$ 24000, 18000 and 8400 subunits were formed when the DNA from plasmid pAN51 was used, only the last two subunits were formed when DNA of plasmid pAN95 was used (figure 7), indicating that the *uncB* gene codes for the $M_r$ 24000 subunit (Downie *et al.* 1981). A new polypeptide which is probably a shortened product of the *uncB* gene was formed when the plasmid pAN95 was used as source of DNA.

To summarize, the relationships between polypeptides of the $F_1$–$F_0$ ATPase and the *unc* genes established so far are: for the $F_1$ ATPase, $\alpha$-subunit *uncA*, $\beta$-subunit *uncD*, $\gamma$-subunit *uncG*, $\varepsilon$-subunit *uncC*; and for the $F_0$ ATPase, $M_r$ 24000 subunit *uncB*, $M_r$ 18000 subunit *uncF*, $M_r$ 8400 subunit *uncE*. 
Assemble of the $\text{F}_1-\text{F}_0$ ATPase

It has been shown that the $\text{uncB}$, $\text{E}$ and $\text{F}$ genes, which code for the subunits of the $\text{F}_0$ ATPase, are the first genes transcribed in the $\text{unc}$ operon and that the other genes which code for the subunits of the $\text{F}_1$ ATPase are read later. This arrangement would be consistent with the idea that the $\text{F}_0$ components are formed first and the products assembled in the membrane to give a complete $\text{F}_0$ ATPase; the $\text{F}_1$ components are then formed and assembled to complete the $\text{F}_1-\text{F}_0$ ATPase complex. Doubts as to the validity of this simple model were raised by observations on one particular mutant (strain AN1007) carrying the $\text{uncD}^{436}$ mutant allele (Cox et al. 1981). Genetic complementation tests showed that this strain was $\text{uncB}^+\text{E}^+\text{F}^+\text{G}^+\text{D}^-\text{C}^-$, indicating a polarity mutation affecting the $\text{uncD}$ gene. The unexpected observation was that washed membranes from strain AN1007, unlike those from normal strains, were impermeable to protons as judged by atebrin fluorescence quenching tests (table 3). Examination of the membrane proteins of strain AN1007 by two-dimensional gel electrophoresis showed that not only were the $\text{F}_1$ subunits absent but the $M_r$ 18000 subunit of the $\text{F}_0$ ATPase was also missing (figure 8). The introduction of the plasmid pAN36 ($\text{uncD}^+\text{C}^+$) into strain AN1007 gave a strain the membranes of which were essentially normal (figure 8). These observations suggested that at least one $\beta$ and/or one $\varepsilon$ subunit had to be attached to the membranes before the $M_r$ 18000 subunit could be incorporated to form a

![Figure 7](image-url)
functional $F_0$. The above results were confirmed with a series of Mu-induced polarity mutants with which it was shown (table 3) that only when a functional $uncD$ gene was present was the $M_r$ 18000 $F_0$ ATPase subunit inserted in the membrane. However, the $M_r$ 18000 subunit was present in membranes from strains carrying polar mutations in the $uncC$ gene, indicating that it was the $\beta$-subunit and not the $\varepsilon$-subunit that was essential.

**Table 3. The effect of washing membranes from the $unc$-436 polar mutant and the presence of the $M_r$ 18000 subunit in membranes from various strains**

(See Cox et al. 1981.)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment of membranes</th>
<th>NADH (fluorescence quenching)</th>
<th>ATP (fluorescence quenching)</th>
<th>$F_0$ ATPase subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN248 ($unc^+$)</td>
<td>—</td>
<td>67</td>
<td>73</td>
<td>+</td>
</tr>
<tr>
<td>AN248 ($unc^+$)</td>
<td>washed</td>
<td>11</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>AN1007 ($uncD436$)</td>
<td>—</td>
<td>90</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>AN1007 ($uncD436$)</td>
<td>washed</td>
<td>87</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>AN1846 (pAN36, $D^+C^+/uncD436$)</td>
<td>—</td>
<td>84</td>
<td>83</td>
<td>+</td>
</tr>
<tr>
<td>AN1303 (Mu::$uncB^+E^+F^+A^+G^-D^-C^-$)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AN808 (Mu::$uncB^+E^+F^+A^+G^-D^-C^-$)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

To see if any of the earlier $F_1$ genes were essential for the formation of a functional $F_0$ ATPase the plasmid pAN36 ($uncD^+C^+$) was inserted into a series of Mu-induced polarity mutants. The results of such experiments indicated that an $\alpha$-subunit was also required for the assembly of a complete $F_0$ ATPase. Examination of the membranes from a series of point mutants by two-dimensional gel electrophoresis and taking into account not only which proteins of the $F_1$-$F_0$ ATPase were present, but also their relative proportions, allowed the formulation of a tentative, but necessarily incomplete, scheme (figure 9) for the assembly of $F_1$-$F_0$ ATPase (Cox et al. 1981). One attractive feature of the proposed assembly sequence is that attachment of the $\beta$- and $\alpha$-subunits before the proton pore is complete could prevent the free flow of protons and avert the potentially disastrous effects of having open proton pores in the cell membrane of a rapidly growing *E. coli*.

**Prospects**

Not much doubt should remain now that *E. coli* can provide an important experimental system in which to study various aspects of the $F_1$-$F_0$ ATPase and to develop a model for energy transduction in higher cells. Determination of the DNA sequence for the $unc$ operon (Gay & Walker 1981a, b; Kanazawa et al. 1981; Mabuchi et al. 1981; Saraste et al. 1981) suggests lines of work on the control of the ATPase complex in growing cells, as well as providing protein chemists with the complete amino acid sequence of all the subunits. No doubt the study of the comparative protein chemistry of the *E. coli* subunits and those from chloroplasts and mitochondria will receive considerable attention. The recent finding that the
Figure 8. Two-dimensional gel electrophoresis of membranes from strains (a) AN1008 (uncD436) and (b) AN1846 (uncD+C+/uncD346). The arrows labelled α, β, γ and δ identify the corresponding subunits of the F$_1$ ATPase. The unlabelled arrows indicate the normal positions of the absent subunits. The ε-subunit was electrophoresed off the gel during the prolonged electrophoresis needed to separate the δ-subunit from a similar polypeptide. The insert shows the high level of β-subunit that remains on the membranes of strain AN1846 after washing of the membranes (Cox et al. 1981).
unc operon also contains a gene coding for a basic protein of unknown function and preceding the \textit{uncB} gene (Gay \& Walker 1981b) will obviously stimulate further work.

The details of the assembly of the \(F_1-F_0\) ATPase will receive further attention. Of particular interest will be whether the assembly in chloroplasts and mitochondria on the one hand and \textit{E. coli} on the other are similar in view of the fact that, in the former, the genes for some \(F_1-F_0\) ATPase components are on the chromosome and others are coded for on the DNA of the organelle.

Although a variety of processes, such as transport across membranes, motility, DNA synthesis, have been studied in various laboratories with use of \textit{unc} mutants (see Downie \textit{et al.} 1979) such experiments have not so far provided really new insights into the mechanism of ATP synthesis. However, I have no doubt that such experiments with \textit{E. coli} will play an important role in elucidating the detailed role of the subunits of the \(F_1-F_0\) ATPase in the synthesis of ATP, particularly when the \textit{unc} mutants used have been characterized to the extent that the nature of the amino acid substitutions in the mutant subunits are known. In addition there are, in any collection of mutants, always a number that, after preliminary examination, have been put in the ‘too hard’ basket because their genetic or biochemical characteristics cannot be explained on the basis of current knowledge. While no doubt the properties of some such mutants will be explained eventually, for example, by there being more than one mutation in the strain, it is the study of the odd mutants that may well provide important clues as to the mechanism of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{assembly_sequence.png}
\caption{Proposed sequence for the assembly of the \(F_1-F_0\) ATPase in \textit{E. coli}. The mutant allele number or strain number indicates the point at which the assembly is interrupted in particular strains. The terminal stages are tentative (Cox \textit{et al.} 1981).}
\end{figure}
ATPase action. It may also be that studies on the biochemistry and genetics of mutants affected in electron transport will give important clues as to the mechanism of ATP formation, as electron transport is an integral part of the overall process of oxidative phosphorylation.

Workers in the field of oxidative phosphorylation over the years have not been noted for their reticence when it comes to theorizing. It is to be hoped that E. coli will help provide the basis for even more theories. Such theories, and debate about them, is to be encouraged provided that the exponents take to heart the comments of Antony van Leeuwenhoek (Dobell 1932) in his letter of 25 December 1700:

'As I aim at nothing but Truth,...if they would expose any Errors in my own Discoveries, I'd esteem it a Service; all the more, because 'twould thereby give me Encouragement towards the Attaining of a nicer Accuracy.'

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References


Dobell, C. 1932 Antony van Leeuwenhoek and his 'little animals'. London: John Bale, Sons and Danielsson.


