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SHORT COMMUNICATION

Transmembrane Respiration-driven H^+ Translocation is Unimpaired in an *eup* Mutant of *Escherichia coli*

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Respiration-driven H^+ translocation has been examined in an *eup* ('energy uncoupled phenotype') mutant of *Escherichia coli* and compared with that observed in its otherwise isogenic wild-type parent. Respiration-driven H^+ translocation was unimpaired in the *eup* mutant strain. It appears that the role of the *eup* gene product lies in the utilization of energized protons pumped across the *E. coli* cytoplasmic membrane.

INTRODUCTION

Certain mutant strains of *Escherichia coli* have been described that, although selected for resistance to the aminoglycoside neomycin, are also rather resistant to colicin K, grow poorly on succinate, and are impaired in the operation of a number of respiration-linked active transport systems (Plate, 1976). Respiratory and ATP hydrolase activities are not decreased in the mutant strains, designated *eup* (for 'energy uncoupled phenotype'), and it was proposed *inter alia* (Plate, 1976) that the *eup* gene codes for a proteinaceous component in the bacterial cytoplasmic membrane that acts to couple the free energy released by respiration to processes such as active transport. Later work (Plate, 1979; Plate & Suit, 1981) showed that the mutant allele maps at 86·5–87 min on the *E. coli* linkage map (Bachmann & Low, 1980), that the *eup* mutation only marginally affects the apparent membrane potential (as judged by the uptake of the methyltriphenylphosphonium cation) and the intracellular pH of these organisms, and that the lesion in some way prevents the mutant strains from coupling energized H^+ movements to solute symport. Physiological and genetic studies are consistent with the view that the *eup* gene is the same as the independently isolated genes *ssd* (Morris & Newman, 1980; Newman *et al.*, 1981, 1982) and *ecfB* (Thorbjarnardóttir *et al.*, 1978).

In view of current controversy concerning the reliability of the methods used for measuring the electrical and pH gradients across the bacterial cytoplasmic membrane (e.g. Kell, 1979; Tedeschi, 1981), we have measured respiration-driven H^+ translocation in an *eup* mutant strain and its otherwise isogenic *eup*$^+$ parent. We report here that there is no significant difference in the ability of the two strains to effect respiration-driven H^+ translocation.

METHODS

Strains. Escherichia coli CJ49 (*lacZ*(Am) *trp*(Am) *eup*$^+$*thi*) and CJ48 (*lacZ*(Am) *trp*(Am) *eup-5 thi*) were generously donated by Dr Charles A. Plate, Northwestern University Medical School, Chicago, U.S.A. and grown aerobically at 37 °C in Ozeki minimal medium supplemented with glucose (0·4%, w/v), thiamin (0·5 μg ml^{-1}) and tryptophan (50 μg ml^{-1}) (Plate & Suit, 1981). After each experiment, strains were checked for their appropriate phenotype by assay for aerobic growth on succinate and sensitivity/resistance to neomycin sulphate (10 μg ml^{-1}).

Respiration-driven H^+ translocation. This was measured by the O_2 pulse method of Scholes & Mitchell (1970). Mid-exponential cultures were harvested, washed three times in 150 mM-KCl containing 0·25 mM-glycylglycine,

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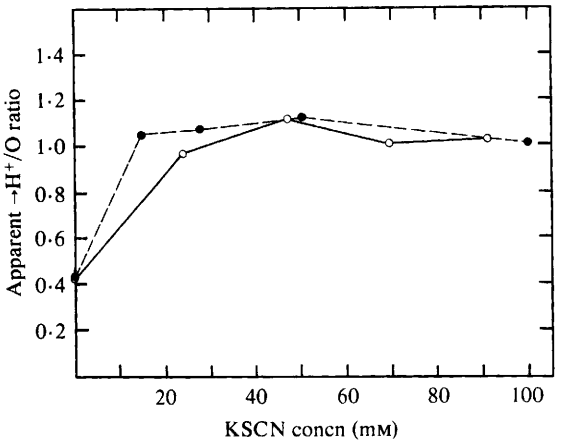

Fig. 1. Respiration-driven H^+ translocation in *E. coli* CJ48 (*eup*) and CJ49 (*eup*$^+$). Measurements were made as described in Methods. The number of cells present, in a final volume of 6 ml, was $1{\cdot}34 \times 10^{10}$ for the *eup* strain (●), and $1{\cdot}74 \times 10^{10}$ for the *eup*$^+$ strain (○). Total cell counts were made microscopically, with an improved Neubauer counting chamber. The amount of oxygen added in each pulse was 23·5 ng-atom, and KSCN was present at the concentrations shown.

pH 6·5, and resuspended at 3 mg dry wt ml^{-1} in the same solution to which carbonic anhydrase at 80 $\mu g\ ml^{-1}$ was added. KSCN was added to the concentrations shown in Fig. 1. Assays were carried out in a vigorously stirred glass reaction vessel maintained at 37 °C and equipped with a sensitive pH electrode connected to a potentiometric system (Kell & Morris, 1980; Hitchens & Kell, 1982). O_2-free N_2 was passed over the surface of the reaction mixture. O_2 pulses, as air-saturated KCl, and calibrating additions of anaerobic KOH and HCl were delivered in the usual way (Scholes & Mitchell, 1970).

Respiration. This was measured with a Clark-type oxygen electrode (Phillips & Kell, 1981).

RESULTS AND DISCUSSION

The apparent $\rightarrow H^+/O$ ratios shown by the *eup*$^+$ and *eup* strains, as obtained by extrapolation of the O_2-induced pH excursions to the half time of O_2 reduction (Scholes & Mitchell, 1970), are shown in Fig. 1. The increase of the apparent $\rightarrow H^+/O$ ratio on the addition of the permeant SCN^- ion (Lawford & Haddock, 1973) demonstrates that the respiration-induced H^+ movements were transmembranous in nature, and not purely scalar (Scholes & Mitchell, 1970). Since the *eup* strain was unable to use succinate, and presumably other respiratory substrates, as carbon and energy source for growth, it was necessary to use glucose-grown cells for these experiments. This explains why the absolute limiting $\rightarrow H^+/O$ stoichiometries were lower than those obtained by other workers using *E. coli* cells grown on respiratory substrates and respiring endogenously (Lawford & Haddock, 1973; Gould & Cramer 1977; Jones, 1977). The crucial observation in the present work is that the ability of the *eup* strain to pump H^+ is unimpaired, in comparison with that of its similarly-grown *eup*$^+$ parent.

We have recently reviewed the available evidence on the mode of action of membrane-active colicins, together with that concerning the nature of the *eup* gene product, and have proposed (Kell & Morris, 1981; Kell *et al.*, 1981) that the normal function of the *eup* gene product is to direct H^+, energized (pumped) by respiration, laterally along the cytoplasmic membrane surfaces between their sources and their sinks such as the H^+-ATPase and H^+-solute symporters. The finding that the *eup* strain is unimpaired in its ability to pump H^+ across its cytoplasmic membrane is consistent with its ability to generate an apparent protonmotive force (Plate, 1979; Plate & Suit, 1981), and, when taken together with the knowledge that it is unable to utilize energized protons (Plate 1976, 1979; Plate & Suit, 1981), allows us to state quite explicitly that efficient protonmotivated energy coupling requires functional proteins additional to the generally recognized reversible proton pumps themselves, a conclusion in line with various earlier proposals (e.g. Plate, 1976, 1979; Plate & Suit, 1981; Hong, 1977; Kell & Morris, 1981; Kell *et al.*, 1981).

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