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Anaerobic Electron Transport in Anaerobic Flagellum Formation in *Escherichia coli*

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Flagellum formation by ubiquinone- and menaquinone-deficient mutant strains of *Escherichia coli* K-12 was studied under both aerobic and anaerobic growth conditions. Ubiquinone was found to be obligatory for aerobic flagellum formation but could be replaced by menaquinone for anaerobic flagellum formation. A mutant devoid of both quinones was immotile aerobically as well as anaerobically. Hence, the respective electron transport system is obligatory for flagellum formation in *Escherichia coli*.

A functional electron transport system was shown recently to be obligatory for flagellum synthesis in aerobically grown *Escherichia coli* (2). Because of the finding of motility in anaerobically grown wild-type cultures (1, 7), the role played by anaerobic electron transport in anaerobic flagellum synthesis was studied here.

E. coli K-12 strains were kindly supplied by I. G. Young. Strains AN384 (*ubiA420 menA401*), AN385 (*ubiA420 men*$^+$), AN386 (*ubi*$^+$ *menA401*), and AN387 (*ubi*$^+$ *men*$^+$) were isogenic transductants, and all were F$^-$ Str^r and possibly Thi$^-$. The *ubiA* and *menA* alleles affect the octaprenyltransferases of the respective pathways of ubiquinone and menaquinone biosynthesis (9, 10). The phenotype of these mutants was confirmed by analyzing both their growth requirements (I. G. Young, personal communication) and their ubiquinone content (2). The UbiA phenotype may be suppressed in the presence of excess *p*-hydroxybenzoic acid (pHBA) in the growth medium.

Bacteria were grown aerobically in 0.8% nutrient broth-0.1 M potassium phosphate buffer (pH 7.0) with continuous gyratory shaking at 30°C and anaerobically in 0.8% nutrient broth-0.1 M potassium phosphate buffer (pH 7.0)-20 mM pyruvate-0.2% nitrate-1 mM thiamine-36 μM uracil-40 μM thymine. Anaerobic cultures were grown in GasPak anaerobic jars (BBL, Division of Becton, Dickinson and Co., Cockeysville, Md.).

Motility was measured by the photographic assay described previously (2). With anaerobic cultures, chloramphenicol (30 μg/ml) was added upon termination of growth. "Motile" cultures were defined as having more than 70% motile bacteria, whereas cultures having less than 1% motile bacteria were termed "immotile." Electron microscopy studies were conducted as described previously (2).

Motility and flagellum synthesis patterns of the four strains grown either aerobically or anaerobically are summarized in Table 1. Flagellum synthesis was not expressed aerobically in the two ubiquinone-deficient strains (AN385 and AN384), confirming our previous results which indicated that aerobic electron transport is required for aerobic flagellum synthesis (2). Surprisingly, by eliminating aerobic electron transport through oxygen deprivation, the *ubiA men*$^+$ strain escaped the inhibition of flagellum expression imposed by a ubiquinone deficiency. On the other hand, the double mutant, devoid of both quinones, was immotile aerobically as well as anaerobically. The motility pattern observed with the *ubi*$^+$ *menA* mutant was reproduced with the double *ubiA menA* mutant in the presence of excess pHBA during growth. Similarly, the motility pattern observed with the wild-type *ubi*$^+$ *men*$^+$ strain was reproduced with the *ubiA men*$^+$ strain in the presence of excess pHBA. It should be pointed out that the motility pattern observed under aerobic growth conditions in the presence of the fortified growth medium (supplemented with pyruvate, thiamine, uracil, and thymine) was similar to the pattern observed aerobically in the absence of supplements in the nutrient broth growth medium. Also, anaerobic growth of AN387, AN385, and AN384 in the presence of 20 mM fumarate instead of nitrate yielded the same motility pattern as observed in the presence of nitrate.

These results point to the obligatory role played by the respective electron transport systems in aerobically and anaerobically grown cultures. Ubiquinone is an obligatory intermediate of aerobic electron transport which cannot be replaced by menaquinone in oxygen reduction (3). The *ubiA* strain grown aerobically is deprived of a functional aerobic electron transport due to ubiquinone deficiency and is also de-

TABLE 1. *Flagellum synthesis and motility patterns of electron transport mutants of E. coli*

Bacterial strain	Growth conditions	Ubiquinone (nmol/mg, dry wt)	Motility	% Bacteria with x no. of flagella/bacterium		Mean no. of flagella/ bacterium
				$0 \leq x \leq 1$	$2 \leq x \leq 7$	
AN387 (*ubi*$^+$ *men*$^+$)	Aerobic	0.54	Motile	28	72	2.8
	Anaerobic	0.39	Motile	26	74	2.6
AN386 (*ubi*$^+$ *menA*)	Aerobic	0.25	Motile	25	75	2.8
	Anaerobic		Motile	18	82	3.6
AN385 (*ubiA men*$^+$)	Aerobic	ND[a]	Immotile	99	1	0.0
	Anaerobic	ND	Motile	27	73	2.6
AN384 (*ubiA menA*)	Aerobic	ND	Immotile	92	8	0.2
	Anaerobic	ND	Immotile	100	0	0.0

[a] ND, Not detectable.

prived of an optional nitrate (fumarate) reductive electron transport system induced solely under conditions of low oxygen pressure and in the presence of the respective acceptor (4, 8). Being deprived of both, the *ubiA* mutant grown aerobically does not express flagellum synthesis. Contrary to the obligatory role played by ubiquinone in oxygen reduction, anaerobic electron transport sustained by either nitrate or fumarate and induced anaerobically is functional with either menaquinone or ubiquinone (5). Anaerobic growth should therefore allow electron transport for the *ubiA men*$^+$ mutant, thus providing an escape from the inhibition of flagellum synthesis imposed aerobically. The double mutant, being devoid of functional electron transport aerobically as well as anaerobically, is thus immotile under all growth conditions.

The correlation observed here between flagellum synthesis and functional electron transport is similar to observations reported recently for phosphate and serine transport in Ubi mutants of *E. coli* (6). Transport was found to be inhibited in *ubiB* mutants grown aerobically and to escape inhibition under anaerobic growth conditions. Phosphate and serine transport was shown not to be affected in *unc* mutants grown aerobically or anaerobically in the presence of fumarate or nitrate (6).

It is premature to speculate at this stage about the mechanism by which flagellum synthesis or assembly is related to functional electron transport. However, the possibility that inhibition of flagellum expression is due to incompetent energization of some specific step in flagellum synthesis may be ruled out in the light of the observation made here with the *ubiA* mutant, which fails to become motile aerobically but does so anaerobically. The requirement for functional electron transport either aerobically or anaerobically seems therefore to reflect repression of flagellum synthesis or assembly under reductive metabolic conditions. Studies to confirm this conclusion will be presented elsewhere.

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