MAPPING OF THE GENE CHL-B CONTROLLING MEMBRAN BOUND NITRATE REDUCTASE AND FORMIC HYDROGEN-LYASE ACTIVITIES IN ESCHERICHIA COLI K 12

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Received March 20, 1970

SUMMARY

Chl r mutants lacking nitrate reductase A and formic hydrogene-lyase have been studied. Chl B maps near met E, its function is probably to code for a protein essential for the formation of "chlorate particle" in anaerobic conditions.

INTRODUCTION

Nitrate reductase A is a membran-bound enzyme (1,2) which has a respiratory function under anaerobic conditions (3). This enzyme can also reduce chlorate to chlorite. Chlorite, or products of its further reduction, are toxic for the cell (4). In presence of chlorate and absence of air, only the mutants which have lost nitrate reductase activity and thus acquired the resistence to chlorate (chl r or chl$^-$) are able to grow (4).

The frequency of spontaneous chl r mutations in E. coli is 10^{-5} to 10^{-6}. Most of these mutations are pleiotropic, resulting in the loss of both nitrate reductase A and formic hydrogenlyase activities (4). Thus, among the chl r mutations obtained in strain PA 601, only 1 % are non pleiotropic mutations, resulting in the specific loss of nitrate reductase, and mapping in the trp region (chl C gene); of the other mutations, which are all pleiotropic, 93 % map in the bio region (chl A and chl E genes) while 6 % map in the mtl region (chl B).

Complementation experiments with cell free extracts of suitable mutants have lead to the in vitro reconstitution of particles whose enzymatic activities are comparable to the activity of wild type strain particles (6). Chl B mutants "complement" with the chl A mutants ;

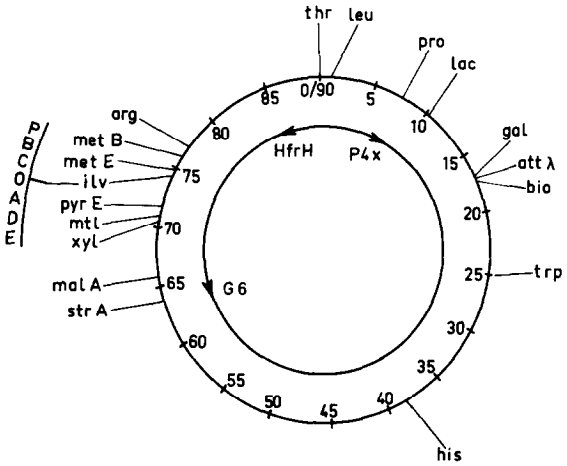

Figure 1 - Chromosomal map of Escherichia coli K 12. The outer circle, with time scale, shows the positions of used markers. The inner circle shows the origins and directions of transfer of the used Hfrs.
Abbreviations : thr : threonine ; leu : leucine ; thi : thiamine ; his : histidine ; pro : proline ; arg : arginine ; ade : adenine ; met : methionine ; ilv : isoleucine-valine ; trp : tryptophane ; pyr : pyrimidine ; gal : galactose ; lac : lactose ; mal : maltose ; xyl : xylose ; mtl : mannitol ; str : streptomycin resistance ; att λ : site of λ attachment.

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hence, these mutants possess in their soluble components the elements necessary for the reconstitution of a complex insoluble structure.

The presence of chl B gene in mtl region has already been reported (6). The present paper describes a more accurate mapping of this mutation on the E. coli chromosome.

MATERIAL AND METHODS

The media used in our experiments were : nutrient broth ; minimal medium of Davis (9) ; E.M.B. medium ; Lennox medium L (10) ; Difco nitrate broth medium (11) ; complete medium with glucose in Durham tubes (12) for the H_2 production.

To measure nitrate reductase and hydrogenlyase activities, methods described by Pichinoty (13, 14) were used.

Genetic localisation was determined by conjugation technic (15) and by transduction with lysates of phage Plkc (10).

Strains are listed in table 1.

Table 1

Strain n°	Polarity	Genotype	Origin
303	Hfr P4x	met	Inst. Pasteur
322	Hfr H	thi	
356	F^-	thr, leu, thi, arg, pro, his, ade; sugars ; str-r	PA601. I. Pasteur
442	F^-	thr, leu, thi, arg, pro, his, ade; sugars ; str-r - chl B^-	356 chl r
448	F^-	thr, leu, thi, arg, pro, ade ; sugars ; str-r ; chl B^-	442 his^+
455	Hfr G6	his	
479	F^-	thr, thi, arg, ilv, gal, mal, xyl, mtl, str-r	PA 3344 trp^+
495	Hfr P4x	chl B	
554	Hfr AT1223	met, pur E	Schaefler
559	F^-	thi, his, met E, ilv D trp, gal, lac, mal, str-r	PA 374
576	F^-	ilv D, met E, trp ; gal,his, lac, mtl, str-r, chl B	495 x 559
578	F^-	ilv D, met E, trp ; gal, lac, mtl, str-r, chl B	576 his^+
583	F^-	met E, trp, gal, lac, mtl, str-r, chl B	455 x 578

EXPERIMENTAL RESULTS.

Estimation of enzymatic activities.

Chl B mutants have lost capacity to reduce nitrate to nitrite and to produce molecular hydrogen from formate produced by degradation of glucose. Measurementsof activities in cell free extracts using Pichinoty's methods (13, 14) give nitrate reductase activities of 30, 75 and 0, 855 µmoles of NO_3^- reduced per mg of protein and per hour, for the wild type strain (356) and a chl B mutant (442) respectively. Hydrogenlyase activity of the mutants chl B is 2 000 times lower than that of wild type strain.

Table 2 - Percent of linkage of unselected markers with selected markers in matings (*) 303 x 442 ; (**) 303 x 576.

Selected markers \ Unselected markers		arg+	met+	mtl+	xyl+	mal+	chl+
(*)	arg+ str-r	-	-	6	6,1	1	50
	mtl+ str-r	35	37	-	83	21,4	96
	xyl+ str-r	-	-	92	-	22	88
	his+ str-r	-	-	-	-	-	6
(**)	mtl+ str-r	-	-	-	-	-	29
	ilv+ str-r	-	88	31	-	-	76
	met E+ str-r	-	-	21	-	-	86

Table 3 - Percent of linkage of unselected markers with selected markers in matings : (*) 455 x 448 ; (**) 455 x 578.

Selected markers \ Unselected markers		str-s	mal+	xyl+	mtl	ilv	met E	arg	thr leu	chl B
(*)	xyl+ his+	-	77	-	72	-	-	31	-	60
	mtl+ his+	61	75	95	-	-	-	-	-	87
	arg+ his+	-	-	48	52	-	-	-	32	80
	thr+ leu+ his+	-	-	-	-	-	-	-	-	69
(**)	met E+ his+	-	-	-	48	97	-	-	-	92
	ilv D+ his+	-	-	-	29	-	70	-	-	63

Matings.

In contrast to chl A and C, chl$^+$ B character is not transmitted to thr$^+$, leu$^+$, pro$^+$ or gal$^+$ recombinants when F$^-$ 442 is mated with Hfr H 322. On the other hand, when matings of the same F$^-$ are carried out with an Hfr of opposed polarity, the P4x, a transfer of the allele chl$^+$ B is observed together with the markers of mtl region. Results summarized in Table 2 show the following transmission gradient : arg - met - chl B - mtl - xyl - mal - .....

Use of Hfr G6 of opposed polarity allowed to precise this mapping ; the analysis of recombinants is indicated in Table 3. These observations and the results of reciprocal matings shown in Table 4 localize the gene chl B in the proximity of met E.

Table 4 - Percent of linkage of unselected markers with selected markers in matings (*) 495 x 356 ; (**) 495 x 559 ; (***) 495 x 479.

Selected markers \ Unselected markers		chl B	mal	mtl	ilv	met E
(*)	mtl$^+$ str-r	96	32	-	-	-
(**)	mal$^+$ str-r	56	-	73	65	61
	mtl$^+$ str-r	74	28,5	-	60	-
(***)	xyl$^+$ str-r	86	-	-	88	-

Transductions.

The frequencies of cotransduction of the gene chl B with different markers of this chromosomal region are reported in Table 5. Among the 15,8 % recombinants met E$^+$ which are selected by ilv D$^+$, none is chl B. Therefore, gene chl B cannot map between these two genes.

Table 5 - Genetic analysis of transductants.

Donor strain	Recipient strain	Selected markers \ Unselected markers	xyl	mtl	pyr E	ilv	met E	chl B	met B
322	442	xyl $^{+}$	-	14,5	-	-	-	<0,1	-
		mtl $^{+}$	14	-	-	-	-	<0,5	-
442	554	pyr E^{+}	1,3	11,2	-	-	-	<0,1	<0,1
		met B^{+}	<0,4	<0,4	<0,4	-	-	<0,4	-
442	479	ilv^{+}	-	-	-	-	-	3,1	-
554	576	ilv D^{+}	-	-	-	-	15,8	<0,1	-
414	583	met E^{+}	-	-	-	-	-	23,8	-
479	583	met E^{+}	-	-	-	8	-	17,4	-

Results are expressed in percents of linkage of unselected markers with selected ones.

Chl B being cotransduced with met E and not with ilv D, we can conclude that the following sequence is the most probable : ilv - met E - Chl B.

DISCUSSION

The gene chl B is probably a structural gene coding for a protein or an enzyme essential for the synthetis of the respiratory particle under anaerobic conditions. If it should be a regulator gene acting on the biosynthesis of nitrate reductase and formic hydrogenlyase, no complementation between mutants chl A and chl B would have been possible in vitro. In the same way, if it should be a gene coding for an intermediate carrier or a cytochrome common for nitrate reductase and formic hydrogenlyase, the extracts from mutants chl B should be active when their nitrate reductase activity is measured using benzyl viologen as artificial electron donor. But the activity of extracts is zero.

The kinetics of complementation shown by Azoulay et al. (6) indicate that there is not the question of a mechanism of activation through incorporation of missing carrier in the case of mutant chl B, and that the reappearance of activity is parallel with the reconstitution of a particulate fraction absent in the mutants.

The product of the gene chl B is not induced by nitrate (6), althrough nitrate reductase is sensitive to this effector. Its real function is not well known, but it is certain that it is essential for the expression of nitrate reductase and formic hydrogenlyase activities, and that it is required for the complementation reaction in vitro with soluble fractions of extracts from the mutant chl A (6). Thus the nitrate reductase activity appears to depend upon the formation of a molecular complex : a "chlorate particle".

ACKNOWLEDGEMENTS

I thank Dr E. Wollman, Dr. Taylor and Dr Schaefler for the strains they have kindly given and Dr. J. Puig whose continuous advice, interest and encouragement made possible the present work.

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