GENETIC MAPPING OF THE *chl* C GENE OF THE NITRATE REDUCTASE A SYSTEM IN *ESCHERICHIA COLI* K_{12}

Juan PUIG, Edgard AZOULAY, Francis PICHINOTY
and Jacqueline GENDRE

Laboratoire de Chimie Bactérienne, CNRS, Marseille 9, France

Received April 25, 1969

Nitrate reductase A is a component of an electron transport system, using NO_3^- as the final acceptor. Inside the cell, the enzyme is associated with the cellular membrane, and it is found in the particulate fractions of cell-free extracts (1, 2). Chlorate reduction which is effected by nitrate reductase A (3), is a reaction lethal to the cell. Therefore, mutants that have defective nitrate reductase are resistant to ClO_3^- (*chl*$^-$) (4).

The high frequency of the mutation *chl*$^+$ → *chl*$^-$ (10^{-5}), reflets the polygenic control of the electron transport system associated with nitrate reductase.

All *chl* mutants lack nitrate reductase activity. Formic-hydrogenlyase activity is lost simultaneously in 99 % of them. The 1 % of *chl* mutants that retain formic hydrogenlyase activity are those altered in the *chl* C gene (5). By mutation of 4 different genes (*chl* A, *chl* B, *chl* D and *chl* E) the pleiotropic phenotype is obtained.

Preliminary experiments (6) indicate that the *chl* C gene is linked to the *trp* region. The present paper reports a more accurate mapping.

RESULTS

By previously described methods (4) we have isolated several *chl*$^-$ mutants derive from strain W 603 *gal*$^-$ *leu*$^-$. The mutant chl_8 has 1/40th the specific activity of nitrate reductase found in the wild type, whereas its formic-hydrogenlyase remains unchanged.

The chl_8 mutant was described as a mutant of the *chl* C gene : M 426 of our collection. A *trp*$^-$ derivative of M 426 was utilized for the

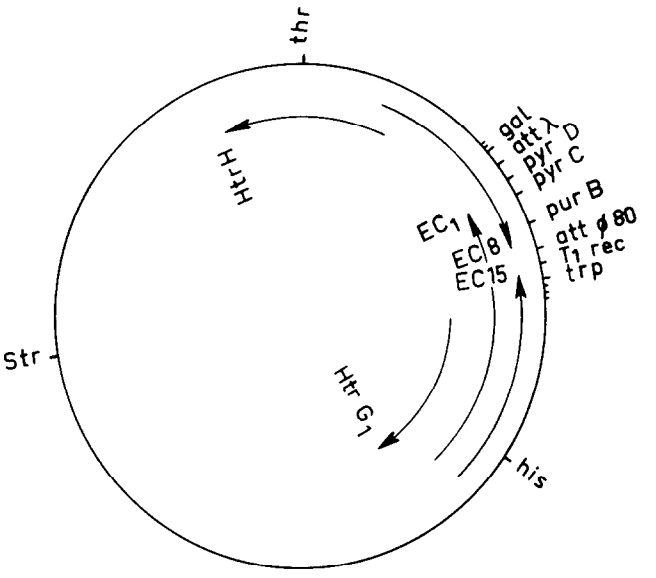

Figure 1 - Origin and direction of transfer of the Hfrs cited (Wollman and Jacob, 1959 (6) ; Signer, Beckwith and Brenner, 1965 (7) ; Beckwith and Signer, 1966 (8).
Abbreviations : *leu* : leucine ; *pyr* : pyrimidine ; *pur* : purine ; *trp* : tryptophan ; *his* : histidine ; *ilv* : isoleucine-valine ; *mtl* : mannitol ; *gal* : galactose ; *str* : streptomycin resistance ; T_1 receptor of phage T_1 ; *Col* V : colicinogenic for colicin V ; *Col* B : colicinogenic for colicin B ; *att* λ : site of λ attachment ; *att* ø 80 : site of ø 80 attachment.

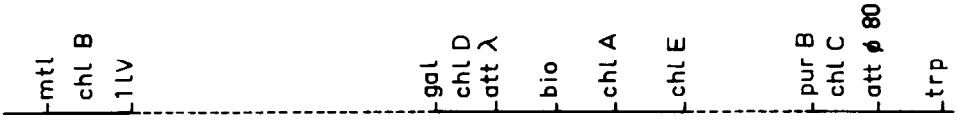

Figure 2 - Map of the *chl* genes in *E. coli* K_{12}

genetic experiments.

Matings were performed with different Hfr strains. Their polarity is indicated in Fig. 1.

By either transduction with ø 80 LFT lysates or sexduction with F' (Col V Col B *trp*) no transmission of chl^+ allele is obtained when *trp* is selected.

Phage P_1 lysates of 426 *chl* C trp^+ strain were used to transduce the trp^+ and pur^+ genes to *pur* B chl^+ trp^- receptor strains. 250 trp^+ transductants and 468 *pur* B were isolated and scored for nitrate and H_2

TABLE I

Percentage of *chl*$^+$ C among recombinants from Hfr *chl*$^+$ x F$^-$ and F$^-$ M 426 *chl* C matings

Hfr Donors	Recombinants		
	leu$^+$ *str*	*gal*$^+$ *str*	*trp*$^+$ *str*
Hfr H	2	18	98
G_6	-	32	85
EC_8	-	40	-
EC_{15}	-	-	0
EC_1	-	-	96

50 to 100 colonies of each type of recombinant from crosses performed using the proportion 1 donor : 20 receptors, were analyzed. NO_2^- and H_2 accumulation were tested in each recombinant (4). The order of transfer of Hfr markers was verified using parallel crosses.

accumulation, under appropriate conditions.

The *chl* C is transmitted to 20 % of *trp*$^+$ transductants and to 1,5 % of *pur* B.

The results of conjugaition experiments (Table I) excluded the possibility that *chl* C was located between *att* 80 and *trp*. This conclusion is confirmed by transduction with ø 80 lysates and by sexduction.

The localization between *pur* B and *att* ø 80 is consistent with all results (Fig. 2).

DISCUSSION

Using the available information (2, 5, 10, 12), we have established the present map (Fig. 2) of the known genes related to the nitrate reduction system. *chl* A, *chl* B and *chl* C have already been described (2, 5, 10). The designation *chl* D has been used for two different genes. Adahya *et al.* call the gene that maps between *gal* and *att* λ, *chl* D (10), and Puig *et al.* give the same designation to the gene mapping between *chl* A and *pur* B. To avoid ambiguity we shall call our former *chl* D (12) *chl* E (Fig. 2).

However, we do not know the exact function of each protein controlled by each gene. Experimental results (2) suggest that membrane-linked nitrate reductase is associated with several others proteins in a parti-

culate complex. At least one of the products (13), that of the *chl* B gene is not induced by nitrate. However nitrate reductase is sensitive to this effector.

The product of the *chl* B gene is essential to nitrate reductase and formic-hydrogenlyase expression, and it is necessary for the *in vitro* complementation reaction, with the soluble fractions of *chl* A and *chl* E mutant extracts (13).

Moreover, a solubilization of cytochrome b_1 has been observed in the mutant *chl* A (14). In the wild type cytochrome b_1 is, to great extent, in particulate form. We interpret this fact not by a derepression of cytochrome b_1 biosynthesis, but as a simple solubilization due to non-formation in the mutant of a macromolecular complex to contain it.

None of the mutants isolated until now can be considered as affecting the structural gene of nitrate reductase. All these arguments lead us to keep the *chl* designation for the different genes that participate in the nitrate reductase A system, rather than using *nar* (14) or NR (15) which refers to the enzyme itself.

ACKNOWLEDGEMENTS

We wish to thank Drs. J.B. Beckwith, P. Frederiq, F. Jacob, L. Pereyra Da Silva, E.R. Signer and E. Wollman for the strains they have kindly given us.

We also thank Dr. D.K. Grossman for helpful discussions.

REFERENCES

1 - IIDA, K. and TANIGUCHI, S., J. Biochem. (Tokyo), *46* (1959) 1041.
2 - AZOULAY, E., PUIG, J. and PICHINOTY, F., Biochem. Biophys. Res. Comm., *27* (1967) 270.
3 - PICHINOTY, F., Bull. Soc. Franc. Physiol. Vég., *12* (1966) 97.
4 - PIECHAUD, M., PUIG, J., PICHINOTY, F., AZOULAY, E. and LE MINOR, L., Ann. Inst. Pasteur, *112* (1967) 24.
5 - PUIG, J. and AZOULAY, E., C.R. Acad. Sci. (Paris), *264* (1967) 1916.
6 - WOLLMAN, E., JACOB, F., "La Sexualité des Bactéries", Masson Ed., Paris, (1959).
7 - SIGNER, E.R., BECKWITH, J.R. and BRENNER, S., J. Mol. Biol., *14* (1965) 153.
8 - BECKWITH, J.R. and SIGNER, E.R., J. Mol. Biol., *19* (1966) 254.
9 - FREDERIQ, P., Personal communication.
10 - ADAHYA, S., CLEARY, P. and CAMPBELL, A., Proc. Nat. Acad. Sci., *61* (1968) 956.
11 - ZYBALSKY, W., Personal communication.
12 - PUIG, J., AZOULAY, E., GENDRE, J., and RICHARD, E., C.R. Acad. Sc. (Paris), *268* (1969) 183.
13 - AZOULAY, E., PUIG, J., COUCHOUD, P., Biochim. Biophys. Acta, *171* (1969) 238.
14 - VENABLES, W.A., GUEST, J., Mol. Gen. Genetics, *103* (1968) 127.
15 - HERRERA, J.R. and DE MOSS, J.A., J. Bacteriol. (in press).