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Locus Determining the Synthesis of δ-Aminolevulinic Acid in *Escherichia coli* K-12

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By using penicillin for selection, Wulff (9) was recently able to isolate some δ-aminolevulinic acid-requiring mutants of *Escherichia coli* K-12. Of 1,500 auxotrophic mutants isolated, only one proved to be δ-ALA$^-$. Preliminary investigations concerning the locus involved in this mutation resulted in its localization in the *pro-thr* segment of the chromosome (9).

δ-Aminolevulinic acid-requiring mutants of *E. coli* K-12 have been isolated also by means of neomycin (5) and have displayed the characters of Ncf$^-$ mutants (normal colony formation deficient; 4). Presumably, these mutants are defective in the first step of heme synthesis; consequently, they may be classed as Hem$^-$ mutants. In contradistinction to the loci affected in the other Hem$^-$ mutants, the *hemA* locus, involved in the synthesis of δ-aminolevulinic acid, could not, however, be cotransduced with the *lac* locus.

A genetic study of one of the δ-ALA$^-$ mutants isolated (SHSP8) showed that the locus affected lies close to the *trp* and *cysB* loci, with which the *hemA* locus proved to be cotransducible. This localization differs from that indicated by Wulff (9).

To study the SHSP8 mutant, we performed mating and transduction experiments. The strains used are listed in Table 1. Conjugation experiments were carried out according to the method described by Wollman and Jacob (8), and mating was interrupted by the method described by the same investigators (7). Transduction by P1kc phage was carried out according to the method of Lennox (3).

The results of these mating experiments are given in Table 2. The *hemA* locus was not injected proximally by the Hfr P4x6 strain, as might have been expected if it were situated in the *pro-thr* segment of the chromosome of *E. coli* K-12. At the same time, control experiments showed that the Hfr P4x6 strain does inject the *pro-thr* segment proximally.

The use of a different donor (Hfr SHSH1) revealed the existence of linkage between the *hemA* and *trp* markers under circumstances in which control matings indicated a normal behavior of the donor strain. To specify the spatial relationship of the *hemA* and *trp* markers, mating experiments were carried out with another *hemA*$^-$ mutant (SHSP18) obtained by transfer of the *hemA8* mutation to a *trp*$^-$ strain. In such experiments, the frequency of linkage between an unselected marker and the selected marker is known to be greater for proximal markers, compared with that of distal markers situated at the same distance. By using a donor injecting the *trp* marker proximally, *trp*$^+$ and *hemA*$^+$ recombinants were selected and analyzed for the frequency of the unselected marker (Table 2). Although, these results seem to indicate a (*gal*), *hemA*, *trp*, (*cysB*) sequence of the markers on the chromosome, they are not very conclusive.

The results of transduction by P1kc phage are given in Table 3. Again, the same type of linkage becomes apparent with the *trp* and *cysB* markers (frequencies of cotransduction 6.4 and 2.8%, respectively). Although the difference in cotransduction frequency between the *trp* and *cysB* markers was not particularly conclusive with regard to the sequence of the *hemA*, *trp*, and *cysB* markers, an analysis of the classes of transductants proved highly significant in this respect (Table 4). In selection for *trp*$^+$, class 2 has a lower probability of appearance (four crossovers) than class 1 (two crossovers) on the assumption of sequence II. However, sequence II seems unlikely, since the frequencies recorded are 4.5% for class 2 and 1.8% for class 1. In selection for *cysB*, the probability of appearance is higher for class 6 (two crossovers) than for class 5 (four crossovers) on the assumption of sequence I; again, the frequencies recorded, i.e., 4.4% for class 6 (1) and 1.5% for class 5, are in agreement with sequence I. The result of reciprocal transduction, with selection for the *hemA*$^+$ marker, points in the same direction. On the assumption of sequence I, class 9 (four crossovers) has a lower probability of appearance than class 10 (two crossovers), whereas on the assumption of sequence II class

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TABLE 1. *Substrains of E. coli K-12 used in the investigation*

No.	Strain	Sex character[a]	Genotype	Origin	Supplied by
1	Hc	Hfr type H	*thi*$^-$		F. Jacob
2	P4x6	Hfr type 2	*met*$^-$		F. Jacob
3	42CH43	Hfr type F[b]	*met*$^-$		P. Fredericq
4	SHSH1	Hfr type F	*arg*$^-$ *his*$^-$ *trp*$^-$ *thi*$^-$ *lac*$^-$ *gal*$^-$ (*col B*)$^+$	Recombination between 42CH43 and PA409	
5	SHSP8	F$^-$	*hemA8 met*$^-$ *str*$^-$	Row	
6	SHSP18	F$^-$	*hemA8 met*$^-$ *trp*$^-$ *lac*$^-$ *str*$^-$	Transduction between P1 (SHSP8) and 84/S	
7	SHSP19	F$^-$	*hemA8 met*$^-$ *lac*$^-$ *str*$^-$	Transduction between P1 (SHSP8) and 84/S	
8	Row	F$^-$	*met*$^-$ *str*$^-$		P. Fredericq
9	PA409	F$^-$	*arg*$^-$ *his*$^-$ *trp*$^-$ *thi*$^-$ *lac*$^-$ *gal*$^-$ *mal*$^-$ *xyl*$^-$ *mtl*$^-$ *str*$^-$		F. Jacob
10	PA6021	F$^-$	*arg*$^-$ *his*$^-$ *leu*$^-$ *pro*$^-$ *thr*$^-$ *trp*$^-$ *purE*$^-$ *thi*$^-$ *lac*$^-$ *gal*$^-$ *mal*$^-$ *xyl*$^-$ *mtl*$^-$ *str*$^-$		F. Jacob
11	84/S	F$^-$	*cysB*$^-$ *met*$^-$ *trp*$^-$ *lac*$^-$ *str*$^-$		P. Fredericq
12	SHS01	F$^-$	*cysB*$^-$ *met*$^-$ *lac*$^-$ *str*$^-$	Transduction between P1 (Row) and 84/S	

[a] The Hfr type is expressed according to Jacob and Wollman (2).
[b] Order of injection of the markers: *xyl, mtl, met ... str, mal* (1).

TABLE 2. *Results of mating experiments*

No.	Donor	Recipient	Selected marker[a]	TE of selected marker[b]	No. of recombinants analyzed	Hfr alleles in recombinants (%)					
						lac	*gal*	*hemA*	*trp*	*cysB*	*his*
1	P4x6	SHSP8	*hemA*$^+$	>60							
2	P4x6	PA6021	*pro*$^+$	6							
3	P4x6	PA6021	*thr*$^+$ *leu*$^+$	17							
4	SHSH1	SHSP8	*hemA*$^+$		116	16.3	30	100	54.3		2.5
5	SHSH1	SHS01	*cysB*$^+$		120		38		92.5	100	4.1
6	Hc	SHSP18	*hemA*$^+$		112	29.4		100	75.8		
7	Hc	SHSP18	*trp*$^+$		117	27.3		82	100		

[a] The counter selected marker was *str*$^-$.
[b] Time of entrance (minutes).

TABLE 3. *Results of transduction by phage P1kc*

No.	Donor	Recipient	Selected marker	No. of transductants analyzed	Donor alleles in transductants (%)		
					hemA	*trp*	*cysB*
1	SHSP8	84/S	*trp*$^+$	110	6.3	100	36.4
2	SHSP8	84/S	*cysB*$^+$	135	5.9	40	100
3	84/S	SHSP19	*hemA*$^+$	140	100	6.4	2.8

11 (four crossovers) has a lower probability of appearance than the classes 9 (two crossovers) and 10 (two crossovers). The actual frequencies recorded were 0.7, 2.1, 4.3, and 92.8% for classes 9, 10, 11, and 12, respectively, affording definite evidence of the occurrence of sequence I. Thus, the results of transduction experiments with P1kc phage confirm the existence of the sequence (*gal*),

TABLE 4. *Classes of transductants obtained in the experiments*

No.	Donor	Recipient	Selected marker	Class of transductants	Class frequency (%)	Possible sequence of markers							
						Sequence I				Sequence II			
						hemA	*trp*	*cysB*	No. of crossovers	*trp*	*cysB*	*hemA*	No. of crossovers
1	SHSP8	84/S	*trp*$^+$	1	1.8	1[a]	1	1	2	1	1	1	2
2	SHSP8	84/S	*trp*$^+$	2	4.5	1	1	0[a]	2	1	0	1	4
3	SHSP8	84/S	*trp*$^+$	3	34.5	0	1	1	2	1	1	0	2
4	SHSP8	84/S	*trp*$^+$	4	59.0	0	1	0	2	1	0	0	2
5	SHSP8	84/S	*cysB*$^+$	5	1.5	1	0	1	4	0	1	1	2
6	SHSP8	84/S	*cysB*$^+$	6 (1)	4.4	1	1	1	2	1	1	1	2
7	SHSP8	84/S	*cysB*$^+$	7 (3)	35.5	0	1	1	2	1	1	0	2
8	SHSP8	84/S	*cysB*$^+$	8	58.5	0	0	1	2	0	1	0	2
9	84/S	SHSP19[b]	*hemA*$^+$	9 (5)	0.7	1	0	1	4	0	1	1	2
10	84/S	SHSP19	*hemA*$^+$	10 (1)	2.1	1	1	1	2	1	1	1	2
11	84/S	SHSP19	*hemA*$^+$	11 (2)	4.3	1	1	0	2	1	0	1	4
12	84/S	SHSP19	*hemA*$^+$	12	92.8	1	0	0	2	0	0	1	2

[a] The donor allele is recorded as 1, and the recipient allele as 0.

[b] With the SHSP8 strain as recipient, the results were less clear-cut, although of the same type: 4.3, 3.5, 6.4, and 85.7% for classes 9, 10, 11 and 12, respectively.

hemA, *trp*, *cysB*, as inferred from mating experiments.

As to the relationship between the *hemA* marker and the su_c and *purB* markers, some approximate inferences may be made by comparing our findings on the frequencies of cotransduction of the markers *hemA-trp* and *hemA-cysB* with the results reported by Signer et al. (6), although the experimental conditions were different. From a comparison of these data, the sequence of the markers under discussion is apparently (*gal*), *purB*, *hemA*, su_c, *trp*, *cysB*, although the sequence su_c, *hemA*, *trp* cannot be excluded.

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