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Mapping of the *hemE* Locus in *Salmonella typhimurium*

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A new type of heme-deficient mutant was isolated in *Salmonella typhimurium* by neomycin selection. The mutant was deficient in uroporphyrinogen decarboxylase activity, coded by the *hemE* gene. The *hemE* gene was located between the genes *rif* and *thi* at 128 min on the chromosomal map of *S. typhimurium*.

To map the so far unidentified *hem* genes in *Salmonella typhimurium*, a great number of heme-deficient mutants selected by neomycin were examined. Only one, designated SAST40, proved to be different for the *hem* mutants described earlier in this microorganism (9-11; M. Desrochers, Ph.D. thesis, University of Montreal, Montreal, Quebec, Canada, 1977). The results of the biochemical study of this mutant are presented here, together with the genetic mapping of the new gene, the *hemE* gene of *S. typhimurium*.

The *hemE* mutant SAST40 used in the present study is derived from the strain *S. typhimurium* SAproC110, which contains an LT2 *pro* segment in an LT7 background. The mutant was isolated by selection with neomycin by the technique described earlier (11). Similar to the other bacterial *hem* mutants (11), SAST40 formed dwarf colonies on usual media. The catalase activity of the mutant, determined by the method of Herbert and Pinsent (4), was nil, suggesting a possible defect in heme biosynthesis.

The capacity of the *hemE* mutant to form porphyrins in the presence of the precursor 5-aminolevulinic acid was examined. The growth medium was brain heart infusion agar (Difco) supplemented with 50 μg of 5-aminolevulinic acid per ml. The cultures were incubated aerobically at 37°C for 72 h. Porphyrins were extracted from the cells by the method described earlier (8), and their yields were calculated by using the molar extinction coefficients recommended by Rimington (6), with the subsequent corrections of Porra and Falk (5). The mutant SAST40 accumulated only uroporphyrin (URO^2) (2,202 nmol/g of dry weight), whereas the parental strain accumulated under the same conditions URO (625 nmol), coproporphyrin ($COPRO^2$) (364 nmol), and protoporphyrin (310 nmol per g of dry weight).

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The mutant accumulated almost exclusively the isomer III of URO, identified by the method of Falk and Benson (3), which indicated that the mutant was affected in the uroporphyrinogen decarboxylase activity (Fig. 1). According to the recently proposed nomenclature, the gene for uroporphyrinogen decarboxylase is designated as *hemE* (8), and hence the *hem* mutation in SAST40 was designated as *hemE1*.

To verify the in vivo results, in vitro synthesis of porphyrins by cell extracts of the mutant SAST40 was investigated by methods described previously (9). The results confirmed those obtained by the in vivo experiments: the extract of the mutant synthesized only URO, whereas that of the parental strain was able to synthesize all three major porphyrins, URO, COPRO, and protoporphyrin. The isomer of URO synthesized in vitro was identified either directly by the method of Falk and Benson (3), as already shown for the experiments performed in vivo, or indirectly, following the decarboxylation of URO to COPRO (2), by the method of Chu et al. (1), which identifies the isomer of COPRO thus obtained. Both methods indicated the presence of the isomer III of URO and of COPRO, respectively. Thus, the defect of the mutant SAST40 is at the level of the uroporphyrinogen decarboxylase and the mutation affects the gene *hemE* (Fig. 1).

Mapping of the *hemE* gene. Mapping of the *hemE* gene was performed by phage P22-mediated transduction, followed by analysis of classes of transductants. The genotypes of the strains used and the results of the analysis of the transductants are given in Table 1. *hemE* is distantly linked (6.6%) to *thi-44* and *purD55*, which cotransduce 100%. The results shown in Table 1 clearly favor a site for it between the genes *rif* and *thi*.

To confirm the location suggested by the results shown in Table 1, the classes of the transductants obtained in the cross P22/SAS550 × SAST40 were analyzed. Three possible gene se-

quences are indicated in Table 2. The distribution of transductants in this cross favors one of the sequences, namely *rif, hemE, thi-44, purD55*.

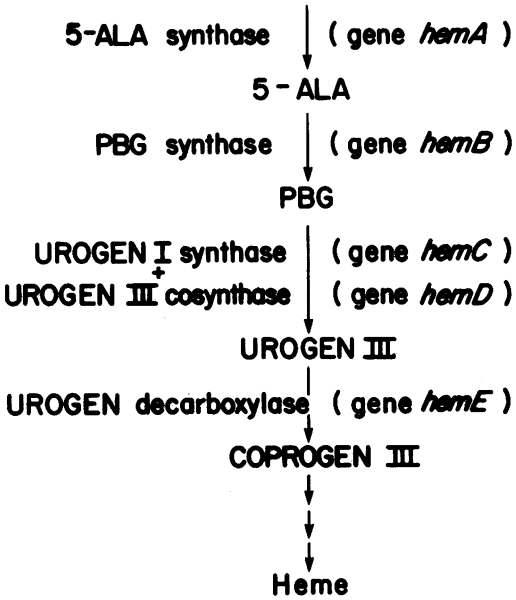

FIG. 1. *Nomenclature of genes involved in early steps in heme synthesis (8). 5-ALA, 5-Aminolevulinic acid; PBG, porphobilinogen; UROGEN, uroporphyrinogen; COPROGEN, coproporphyrinogen.*

This suggests that the *hemE* gene is located between the genes *rif* and *thi* at 128 min on the chromosomal map of *S. typhimurium* (7). This is the first identification of the *hemE* gene in *S. typhimurium* and its location corresponded to one of the two locations suggested in *Escherichia coli* K-12, where the analysis of classes of transductants gave no clear-cut results (8).

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TABLE 1. *Mapping of the hemE locus by P22-mediated transduction*

Donor		Recipient		Selected marker	No. of transductants analyzed	Donor alleles in transductants (%)				
Designation	Genotype	Designation	Genotype			*argF*	*rif*	*thi*	*purD*	*metA*
SAS1601	*argF118 thiA36*	SAST40[a]	*hemE1 proC110*	*hemE*$^+$	111	0.0		26		
SA572	*metA22 trpB2 hisF1009 xyl-1 ilvA99 pyrE231 malB111 strA201*	SAST40	*hemE1 proC110*	*hemE*$^+$	90					4.5
SAS550[b]	*thi-44 purD55 rif*^{r}	SAST40	*hemE1 proC110*	*hemE*$^+$	503		13.5	6.6	6.6	
SA572	*thi-44 purD55 rif*^{r}	SAthi-44	*thi-44 purD55*	*purD*$^+$	116			100	100	25
SAthi-44	*thi-44 purD55*	SA572	*thi-44 purD55*	*metA*$^+$	113			11.5	11.5	100

[a] *hemE1* mutation in SAproC110 (LT2 *pro* segment in LT7).
[b] *rif*^{r} mutation in SAthi-44 (*thi-44 purD55*).

TABLE 2. *Classes of hem*$^+$ *transductants obtained in the cross P22/SAS550 × SAST40*

Class of transductants		No. of transductants	No. of crossovers if the sequence is:		
Designation	Nonselected markers		*hemE rif thi-44 purD55*	*rif hemE thi-44 purD55*	*rif thi-44 purD55 hemE*
1	*rif*^{s} *thi*$^+$ *purD*$^+$	410	2	2	2
2	*rif*^{r} *thi*$^+$ *purD*$^+$	58	2	2	4
3	*rif*^{s} *thi purD*	25	4	2	2
4	*rif*^{r} *thi purD*	10	2	2	2

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