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Chromosomal Location of a Gene for Chemical Longevity of Messenger Ribonucleic Acid in a Temperature-Sensitive Mutant of *Escherichia coli*

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Genetic analysis of a thermolabile mutation affecting alteration of messenger ribonucleic acid stability indicates that the gene *ams* maps close to *pyrC* gene (23 min) on the *Escherichia coli* chromosome and has a cotransduction frequency of 29.6% with *pyrC* gene. The probable gene order is *pyrD-ams-pyrC-purB*.

We have previously described a temperature-sensitive *Escherichia coli* mutant with a longer chemical lifetime of mRNA. At the nonpermissive temperature, chemical degradation of mRNA species is significantly slowed in the mutant (5). It was also confirmed that a thermolabile mutation in the mutant is responsible for alteration of the mRNA lifetime from revertant test to temperature-resistant growth (7, 8). We proposed the name *ams* for the gene that is responsible for alteration of mRNA stability (7, 8).

To genetically map the *ams* gene, various Hfr strains were crossed with a temperature-sensitive strain, HAK117 (Pro^- Ams^-), or with its derivative, HAK119 (Thy^- Ams^-). The temperature-sensitive mutation of HAK117 was previously shown to be responsible for the observed alteration in lifetime of mRNA species (8). The genotypes of all strains employed in this study are listed in Table 1. The mutant strain was first crossed with two Hfr strains, CSH61 (transfer order: o-*purE-lac*-...) and CSH77 (o-*cheC-his*-...), and none of 50 Pro^+ Str^r or Thy^+ Str^r recombinants in both crosses showed temperature-resistant growth (Table 2). It was therefore indicated that the temperature-sensitive mutation was roughly located between *purE* gene and *his* gene on the *E. coli* chromosome (2). Then, to obtain a finer map position of the *ams* gene, HAK117 was crossed with KL96 (o-*his-aroH*-...) or with KL208 (o-*rac-trp*-...). Pro^+ Str^r recombinants were selected in both crosses. Three of 36 recombinants in the former cross (KL96 × HAK117) and 79 of 100 recombinants (KL208 × HAK117) were found to be temperature-resistant clones, respectively (Table 2). In addition, among 50 (Thy^+ Str^r) recombinants in the cross of CSH69 (o-*pyrC-trp*-...) × HAK119, 7 proved to be temperature-resistant clones. It was thus suggested that the *ams* gene is closely linked to the *pyrC* (23 min)-*trp* (27 min) region.

P1 transduction was carried out to focus on markers around *pyrC-trp*. A preliminary transduction test with P1*vir* phage showed close linkage of the *ams* gene to the *pyrC* gene, but not to the *trp* gene. P1 grown on HAK117 (*pyrC*$^+$ *purB*$^+$ *ams*) was used to transduce CS101-2U5 (*pyrC ams*$^+$) or H680 (*purB ams*$^+$), and each Pyr^+ and Pur^+ transductant (respectively) was analyzed. Among 304 Pyr^+ transductants selected from the former cross (HAK117 × CS101-2U5), 90 proved to be *ams* (Table 3). In contrast, the latter cross (HAK117 × H680) yielded, out of 804 Pur^+ transductants selected, 5 that were found to show temperature-sensitive growth (Table 3). In addition, we examined the relative linkage of the *pyrC* gene to the *purB* gene (25 min) by using CS101-2U5 (*pyrC purB*$^+$) as donor and H680 (*pyrC*$^+$ *purB*) as recipient (*pyrC* and *purB* are at 23 min and 25 min, respectively) (2). Among 817 Pur^+ transductants from the cross CS101-2U5 × H680, 10 were found to be *pyrC* clones (Table 3). It is therefore indicated that the *ams* gene is closely linked to *pyrC* gene with a cotransduction frequency of 29.6% and also that the gene is located on the *pyrD* (21

TABLE 1. *Bacterial strains employed in this study*

Strain	Genotype	Source (reference)
CSH61	Hfr *trpR thi*	CGSC[a]
CSH69	Hfr *thi*	CGSC
CSH77	Hfr	CGSC
KL96	Hfr *thi rel*	CGSC
KL208	Hfr	CGSC
HAK117	F^- *thr leu proA argE rpsL gal ams*[b]	(8)
HAK119	as HAK117, but *thy*, trimethoprim selection	This study
CS101-2U5	Hfr *pyrC metB relA tonA*	CGSC
H680	F^- *purB trp his tyrA thi lacY gal mal xyl mtl rpsL tonA tsx supE*	CGSC

[a] From B. Bachmann, *Escherichia coli* Genetic Stock Center, Yale University, New Haven, Conn.

[b] *ams* is a gene in which a temperature-sensitive mutation with altered lifetime of mRNA occurs (7, 8).

TABLE 2. *Mating experiment of various Hfr strains with HAK117 (or HAK119) carrying altered lifetime of mRNA*

Donor	Transfer order[a]	Recipient	Selected markers	No. of colonies	*ams* genotype[b]	
					+	−
CSH61	(o-*purE-lac*-...)	HAK117	Pro$^+$ Str^r	50	0	50
CSH77	(o-*cheC-his*-...)	HAK119	Thy$^+$ Str^r	50	0	50
KL208	(o-*rac-trp*-...)	HAK117	Pro$^+$ Str^r	100	79	21
KL96	(o-*his-aroH*-...)	HAK117	Pro$^+$ Str^r	36	3	33
CSH69	(o-*pyrC-trp*-...)	HAK119	Thy$^+$ Str^r	50	7	43

[a] See Miller (6).

[b] *ams* genotype was tested if colonies could be grown on L-broth agar at 42°C.

TABLE 3. *Transduction mapping of ams gene in strain HAK117*[a]

Donor	Recipient	Selected	Unselected markers: transductants/total (%)
HAK117	CS101-2U5	*pyrC*$^+$	*ams*: 90/304 (29.6)
HAK117	H680	*purB*$^+$	*ams*: 5/804 (0.6)
CS101-2U5	H680	*purB*$^+$	*pyrC*: 10/817 (1.2)

[a] Transduction with P1*vir* phage was carried out as described previously (9).

min) side of *pyrC* gene rather than on the *purB* (25 min) side. The map order indicated is *pyrD-ams-pyrC-purB*.

Several genes for RNases have been mapped so far on the recalibrated *E. coli* chromosome (2): RNase I (*rna*) (11), RNase II (*rnb*) (9), and RNase III (*rnc*) (1, 12), at 14, 28, and 55 min, respectively. In addition, the gene (*pnp*) for polynucleotide phosphorylase is located at 68 min (10). Although it was recently suggested that polynucleotide phosphorylase was involved in mRNA turnover in heat-shocked *E. coli* cells with altered 30S ribosomal subunits (3), the *ams* gene is apparently separable from the *pnp* gene as well as from the *rna*, *rnb*, and *rnc* genes.

Whereas the temperature-sensitive mutation in the *ams* gene retards chemical degradation of mRNA, the mutation appears not to alter the functional lifetime of messages for β-galactosidase, tryptophan synthetase, or bulk proteins (5, 8). We therefore suggest that the *ams* gene might be involved in an earlier step in the chemical decay of mRNA molecules, (4, 8).

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