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Genetic Map Location of the *Escherichia coli dnaG* Gene

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The *dnaG* locus of *Escherichia coli* K-12 has been mapped at about 60 min on the genetic map by three-factor crosses using P1 transduction. In crosses selecting for *dnaG*$^+$, the cotransduction frequency with the *tolC* marker is 15% and that with the *uxaC* marker is 49%. The gene order is *tolC dnaG uxaC*.

The *dnaG* locus (8) is one of at least four loci whose gene products are needed for the elongation reactions of deoxyribonucleic acid replication. Lark (4) was the first to suggest that the *dnaG* gene product regulated the initiation of Okazaki piece synthesis, and Louarn (5) presented further data supporting this view in a paper analyzing the molecular polarity and size distribution of Okazaki pieces derived from a *dnaG* lambda lysogen.

The *dnaG* protein has been purified by in vitro complementation assays (3, 11) and appears to be a novel ribonucleic acid polymerase (1). There is some evidence suggesting that Okazaki pieces are initiated by short ribonucleic acid primers (7). Thus, the biochemical properties of the protein fit nicely with the view that the protein is, indeed, at least partly responsible for the initiation of Okazaki piece synthesis.

In contrast to this progress on the biochemical front, the genetic analysis of the *dnaG* locus has been hampered by a paucity of nearby markers which could be used for mapping studies. In their initial study of the locus, Wechsler and Gross (8) noted that *dnaG* mutants could be complemented by the wild-type *dnaG*$^+$ allele carried on F'102 and that temperature resistance was transferred as an early marker in crosses with Hfr's AB312 and AB313 but not with P13. These results suggested that the *dnaG* locus mapped in the region from about 59 to 63 min, but Wechsler and Gross failed to detect linkage by P1 transduction with any of the markers *argG*, *metC*, *strA*, or *spc*.

Recently, several new loci have been identified in this region of the map, and we were encouraged to reexamine the problem of mapping the *dnaG* locus by use of P1 transductions. We have found a linkage of the *dnaG* gene to both the *tolC* locus, which determines resistance or sensitivity to colicin E1 (10), and the *uxaC* locus, which is the structural gene for uronic isomerase (6), one of the principal enzymes of the hexuronate pathway. *uxaC* mutants are unable to grow on plates containing glucuronate or galacturonate as the principal carbon source.

The data in Table 1 show the result of a three-factor cross between strain PC3 (relevant markers, *tolC*$^+$ *dnaG3 uxaC*$^+$) and strain PB3 (relevant markers, *tolC dnaG*$^+$ *uxaCl*). Summing the appropriate data in the last column for the cross using PB3 as the donor and PC3 as the recipient gives a cotransduction frequency for *dnaG tolC* of 15% and for *dnaG uxaG* of 49%. Using the data from both crosses and assuming the recombinants requiring four crossovers would appear among the progeny less frequently than those requiring two crossovers, we can conclude that the gene order is *tolC dnaG uxaC*. Since *tolC* is transferred as a late marker by Hfr KL14 (2), which presumably shares a common transfer origin with Hfr AB312, this order is consistent with the observation noted above, i.e., that *dnaG* is transferred as an early marker in AB312 crosses, only if we suppose that the origin of AB312 is between *tolC* and *dnaG*. It is also clear that, since the cotransduction frequency of *uxaC* and *argG* is only 2% and the cotransduction frequency of *tolC* and *metC* is only 6% (6), this gene order would imply little, if any, detectable cotransduction between *dnaG* and *argG* or *metC*, in agreement with the failure of Wechsler and Gross to find such cotransduction.

In view of the rather high cotransduction frequency of *tolC* and *dnaG* and the ease with which the *tolC* marker can be inserted into strains by selecting for mutants which are both resistant to colicin E1 and sensitive to deoxycholate (10), it should now be relatively easy to transfer *dnaG* mutations between strains for the purpose of further study of this intriguing locus.

TABLE 1. *Order of the tolC, uxaC, and dnaG markers by P1virA transductions*[a]

Donor	Recipient	Selected marker	Distribution of unselected markers[b]		
			Classes	No.	Frequency (%)
PC3 (*tolC*$^+$ *dnaG3 uxaC*$^+$)	PB3 (*tolC dnaG*$^+$ *uxaC1*)	*tolC*$^+$[c]	*dnaG*$^+$ *uxaC*$^+$	2	2
			dnaG$^+$ *uxaC*$^-$	63	66
			dnaG$^-$ *uxaC*$^+$	2	2
			dnaG$^-$ *uxaC*$^-$	28	29
PB3 (*tolC dnaG*$^+$ *uxaC1*)	PC3 (*tolC*$^+$ *dnaG3 uxaC*$^+$)	*dnaG*$^+$[d]	*uxaC*$^+$ *tolC*$^+$	55	43
			uxaC$^+$ *tolC*$^-$	11	9
			uxaC$^-$ *tolC*$^+$	55	43
			uxaC$^-$ *tolC*$^-$	8	6

[a] The P1 techniques were essentially those of Wehr et al. (9).

[b] The unselected markers *dnaG*, *tolC*, and *uxaC* were scored by replica plating patch plates of recombinants to either L plates at 40 C, L plates containing 1% deoxycholate at 30 C, or M9 minimal plates containing galacturonate 0.2% as the principal carbon source at 30 C, respectively.

[c] *tolC* mutants are sensitive to deoxycholate (10). *tolC*$^+$ transductants were selected on L plates containing 0.25% sodium citrate. After 4 h at 30 C, the cells were overlaid with 2 ml of L top agar containing 1% deoxycholate. Transductants were scored after 3 days at 30 C.

[d] Temperature-resistant transductants were selected on L plates containing 0.25% sodium citrate. After 3 h at room temperature (22 to 25 C), the plates were shifted to 40 C. Transductants were scored after 2 to 3 days.

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