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Genetic Analysis of *acrA* and *lir* Mutations of *Escherichia coli*

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An analysis of *acrA* (acriflavine- and methylene blue-sensitive) and *lir* (lincomycin- and erythromycin-sensitive) mutants of *Escherichia coli* indicated that these mutations are probably within the same gene.

Mutations in the *acrA* gene of *Escherichia coli* confer several phenotypic traits upon a strain, including sensitivity to acridine orange and methylene blue dyes (3, 4, 6). The *acrA* locus was assigned a map position at min 10 between *tsx* (min 9) and *purE* (min 12) on the basis of P1 phage transduction experiments (4, 6). *lir* strains of *E. coli* have been isolated as lincomycin- or erythromycin-sensitive mutants. Interrupted-mating experiments suggested that the *lir* locus was at a map position clockwise of the *lac* operon (min 8) (1). In an effort to identify genes that are near the *dnaZ* and *dnaX* loci at min 10.4 to 10.5, we tested λ *dnaZ*$^+$ transducing phages (7) for their ability to complement *acrA* and *lir* mutations. These mutations were not separated by any of the phages. Two phages transduced *acrA*$^+$ (methylene blue insensitivity) to an *acrA* recipient and *lir*$^+$ (lincomycin insensitivity) to a *lir* strain; six other λ *dnaZ*$^+$ phages carried neither *acrA*$^+$ nor *lir* (Table 1). This identical pattern of transduction of *lir*$^+$ and *acrA*$^+$ by these phages suggested that *lir* and *acrA* might be alleles. Evidence in support of this conclusion was provided by the finding that an *acrA* mutant (strain JE16) was sensitive to low levels (100 to 200 μg/ml) of lincoymcin and erythromycin, and that all three of the *lir* strains tested (N33, N34, and N35) were sensitive to methylene blue (50 μg/ml). Furthermore, spontaneous *acrA*$^+$ revertants of an *acrA* strain (JE16) and spontaneous *lir*$^+$ revertants of three different *lir* strains (N33, N34, and N35) became insensitive to both lincomycin and methylene blue. These data, and our inability to separate the *lir* and *acrA* genes by transduction with λ *dnaZ*$^+$ phages, indicate that *acrA* and *lir* mutations are probably within the same gene.

The order of genes in the *acrA* (*lir*) region is *tsx acrA* (*lir*) *dnaZ dnaX adk purE* (unpublished data). Nakamura and Sugamura have reported that the *acrA* gene product is a membrane protein (3) which may interact with adenylate kinase (4), the product of the *adk* gene and perhaps a membrane protein also (2). The facts that *acr* (*lir*) mutations increase sensitivity to several agents and that *acr* protein is found in the membrane suggest that mutations in this gene directly or indirectly increase the entry of these agents into cells.

Membrane protein alteration by the *acr* (*lir*) mutation could explain the observation by Apirion (1) that ribosomes prepared from *lir* mutants were more sensitive to lincomycin and erythromycin in protein synthesis in vitro than were ribosomes from *lir*$^+$ strains. As ribosomes are known to interact with membranes (5), it is possible that the presence of altered membranes in the ribosome preparations from *lir* mutants could have accounted for those results (1).

TABLE 1. Transduction tests with λ*dnaZ*$^+$ phages[a]

Transducing phage	*E. coli* recipient[b]		
	AX727 (*dnaZ*[Ts])	JE16 (*acrA*)	N35 (*lir*)
λ *dnaZ*$^+$ 2	+	−	−
λ *dnaZ*$^+$ 17	+	−	−
λ *dnaZ*$^+$ 18	+	−	−
λ *dnaZ*$^+$ 6	+	−	−
λ *dnaZ*$^+$ 11	+	−	−
λ *dnaZ*$^+$ 20	+	−	−
λ *dnaZ*$^+$ 14	+	+	+
λ *dnaZ*$^+$ 38	+	+	+

[a] Phages were tested for transduction by spotting lysates onto plates spread with 10^8 recipient cells. For *dnaZ*$^+$ transduction, the procedure was that described by Walker et al. (7). For *acrA*$^+$ and *lir*$^+$, plates containing 50 μg of methylene blue per ml (for *acrA*$^+$) or 100 μg of linomycin per ml (for *lir*$^+$) were incubated for 24 h at 37°C. Transduction was evident by growth of the recipients in the presence of the inhibitor. +, Positive; −, no transduction.

[b] Recipients were made lysogenic with λ$^+$.

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