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 lincomycin 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The order of genes in the *acrA* (*lir*) region is *tsx* *acrA* (*lir*) *dnaZ* *dnaX* *adk* *purE* (unpublished data).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

<table>
<thead>
<tr>
<th>Transducing phage</th>
<th>E. coli recipient&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>dnaZ</em>&lt;sub&gt;TS&lt;/sub&gt;</th>
<th>JE16 (acrA)</th>
<th>N35 (lir)</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 17</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 11</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 20</td>
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<td>+</td>
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<tr>
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<tr>
<td>λ <em>dnaZ</em>&lt;sup&gt;+&lt;/sup&gt; 38</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Phages were tested for transduction by spotting lysates onto plates spread with 10<sup>6</sup> recipient cells. For *dnaZ* transduction, the procedure was that described by Walker et al. (7). For *acrA*<sup>+</sup> and *lir*<sup>+</sup>, plates containing 50 μg of methylene blue per ml (for *acrA*<sup>+</sup>) or 100 μg of lincomycin per ml (for *lir*<sup>+</sup>) 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.

<sup>b</sup> Recipients were made lysogenic with λ<sup>+</sup>.

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