Gene Transfer between Related Bacteria by Electrotransformation: Mapping Salmonella typhi Genes in Salmonella typhimurium

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Typhoid fever is a complex systemic infection quite widespread in developing countries (17). Since its causative agent, Salmonella typhi, is a strictly human pathogen, it has been difficult to find an appropriate animal model for virulence studies. This limitation is partially overcome by the use of human cell lines for in vitro studies. Similar to the results obtained for other bacterial pathogens, this approach is beginning to yield information on the genes involved in invasion and other virulence determinants (3, 4, 7).

Analysis of the S. typhi chromosome has revealed some major differences with respect to the closely related bacteria Salmonella typhimurium and Escherichia coli. Most notable is the inversion of large chromosomal segments thought to result from recombination between rRNA loci (11–13). In spite of these differences, the gene order within the inverted regions and elsewhere in the chromosome is virtually the same in S. typhi as in S. typhimurium and the two bacterial serovars share more than 90% homology at the DNA sequence level (5). Indeed, segments of the S. typhimurium chromosome can undergo recombination with the homologous region in the S. typhi chromosome once the natural barrier imposed by the mismatch repair system is eliminated by mutation (mutS) in the recipient strains (22, 23).

A major problem encountered in the genetic analysis of S. typhi is the lack of a convenient gene transfer system and, in particular, of a generalized transducing phage comparable to phage P22 of S. typhimurium. While P22 will deliver DNA into S. typhi, making S. typhimurium-S. typhi crosses possible (22, 23), it is incapable of multiplying inside this host, thus preventing gene transfer in the opposite direction, i.e., from S. typhi to S. typhimurium, or between S. typhi strains. To try to circumvent this problem, we sought to test whether S. typhi genetic material introduced into S. typhimurium cells by electrotransformation could undergo homologous recombination.

Transformation of S. typhimurium with linear DNA. As a preliminary test of the method we used a cloned DNA fragment from S. typhimurium as input material. The DNA insert of plasmid pCV47 contains the entire S. typhimurium leucine operon plus approximately 13 kb of neighboring DNA (20). This insert is released by BamHI treatment as an 18.5-kb DNA fragment. pCV47 DNA, either cleaved with BamHI or untreated, was used to transform S. typhimurium strains in which the leu4 gene was inactivated by a MudI insertion. Three recipient strains were compared: MA2290, which expresses a functional RecBCD enzyme; MA5133, in which the nuclease activity of RecBCD (Exo V) is inactivated (39); and MA5031, which harbors a recBD deletion but is recombinant proficient due to an sbc mutation (sbcE218 [8]). Results shown in Table 1 confirm that, as suggested from previous work with E. coli (18), inactivation of Exo V in S. typhimurium greatly improves the recovery of transformants with linear DNA. Leu+ transformants were found to be kanamycin sensitive and Lac−, consistent with their resulting from recombination events which replace the leuA::MudI insertion with the wild-type leuA gene. The higher transformation efficiency in TABLE 1. Transformation of S. typhimurium with plasmid DNA

<table>
<thead>
<tr>
<th>Strain</th>
<th>rec genotype</th>
<th>No. of transformants/10⁷ CFU/μg of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Circular DNA</td>
</tr>
<tr>
<td>MA2290</td>
<td>rec⁠</td>
<td>3.1 × 10⁷</td>
</tr>
<tr>
<td>MA5133</td>
<td>recD</td>
<td>2.1 × 10⁷</td>
</tr>
<tr>
<td>MA5031</td>
<td>recBD sbcE</td>
<td>3.2 × 10⁷</td>
</tr>
</tbody>
</table>

Strains are derivatives of S. typhimurium LT2. Some strains derive from strains described in references 8 and 9; they were constructed by P22-mediated transduction (as previously described [14]). Genotypes are as follows: MA2290, leu-3243 leuA3241::MudI; MA5133, leu-3243 leuA3241::MudI recD545::Tn10dTc; MA5031, ΔsbcE218 ΔargK-recBD1742 sbcE21 f68157:Tn10dTc.

Electrotransformation was carried out with a Bio-Rad Gene Pulser. Bacteria were made competent by procedures recommended by the manufacturer. Plasmid DNA, purified by equilibrium centrifugation in a CsCl-ethidium bromide gradient (14), was mixed into the bacterial cell suspension (50 μl) in a chilled cuvette (0.2-cm electrode gap). A single pulse of 12.5 kV/cm (2.5 kV, 200 μF) was applied, and 1 ml of prewarmed SOC medium (2% Bacto Tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) was immediately added. The bacteria were transferred to glass tubes and shaken for 1 h at 37°C prior to plating onto selective medium. Data are from one representative experiment. The number of transformants is expressed per microgram of DNA per 10⁷ viable CFU at the end of the recovery period.

Untreated pCV47 DNA (prepared from S. typhimurium; 25 ng per transformation). Leu+ transformants were selected on minimal E medium (14). More than 99% of transformants were Amp̲ and Lac−.

pCV47 DNA cleaved with BamHI (25 ng per transformation). Leu+ transformants were selected as described for circular DNA.
The recBD sbeE background is indicative of the “hyper-rec” phenotype of this strain (8, 9).

We then evaluated the ability of S. typhimurium to yield Leu<sup>+</sup> recombinants when transformed with bulk chromosomal DNA prepared from S. typhimurium or S. typhi. Results in Table 2 show that this is indeed possible provided the nucleotide activity of RecBD of the recipient strain is inactivated. Again, the frequency of recombination is higher in the sbeE21 background. In addition, in the experiments involving S. typhi DNA, the formation of Leu<sup>+</sup> recombinants also requires that the recipient strain be defective in mismatch repair (mutS). This is in agreement with the known inhibitory effect of the mismatch repair system on recombination between closely related sequences (22, 23).

In a separate experiment, chromosomal DNA from an S. typhi strain carrying a leuA::MudJ insertion (constructed by P22 transduction [22]) was used to transform an S. typhimurium recipient containing an intact leu operon region (MA5100). Kanamycin-resistant (Kan<sup>+</sup>) recombinants were selected. Although such isolates occurred at a somewhat lower frequency than the Leu<sup>+</sup> recombinants described above, the effect of recD and mutS was nearly identical to the data in Table 2 (data not shown). Southern analysis confirmed that the structure of the leu operon region in four independent Kan<sup>+</sup> transformants was indistinguishable from that of donor DNA (data not shown). Thus, no unusual rearrangements accompanied the acquisition of the MudJ insertion by the recipient strain.

Although the mutS mutation makes genetic exchanges between S. typhi and S. typhimurium possible, the frequencies with which recombinants were recovered in the above experiment are low. We sought to see whether the efficiency of the process could be increased by improving the transformation step. S. typhimurium SLA213 (met<sup>A22</sup> metE551 galE496 rpsL120 sly104 Δ[Fes1] H1-b H2-ε,n x nml hsdL6 hsdS29) was previously recognized to be a particularly suitable transformation recipient owing to galE and hsd mutations that favor DNA uptake and lower the restriction barrier, respectively (16). Upon repeating the above experiments with the SLA213 background, we observed a 10-fold increase in the efficiency of recovery of recombinant clones provided that recD and mutS mutations were both present (strain MA5383, see Table 3, footnote a). Such an improvement was not specific to the leu operon region but was also observed in exchanges involving the his operon and the proU operon (data not shown). Unfortunately, limitations in the availability of selectable markers hampered the construction of the triply mutated SLA213 derivative carrying the recBD deletion sbeE21 and mutS::Tn10Dcm. We therefore adopted strain MA5383 (Table 3) for the mapping experiments described below.

**Mapping of S. typhi mutations in S. typhimurium.** The possibility of moving mutations isolated in S. typhi to S. typhimu-

### TABLE 2. Transformation of S. typhimurium with chromosomal DNA

<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>rec mut</th>
<th>No. of transformants/10&lt;sup&gt;8&lt;/sup&gt; CFU&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S. typhimurium</th>
<th>S. typhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA2290</td>
<td>rec&lt;sup&gt;+&lt;/sup&gt; mut&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MA5114</td>
<td>rec&lt;sup&gt;-&lt;/sup&gt; mutS</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>MA5133</td>
<td>recD mut&lt;sup&gt;+&lt;/sup&gt;</td>
<td>12</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>MA5116</td>
<td>recD mut&lt;sup&gt;-&lt;/sup&gt;</td>
<td>21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MA5031</td>
<td>recBD sbeE mut&lt;sup&gt;+&lt;/sup&gt;</td>
<td>41</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MA5145</td>
<td>recBD sbeE mutS</td>
<td>17</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Strains MA5114, MA5116, and MA5145 were derived from S. typhimurium strains MA2290, MA5133 and MA5031, respectively (Table 1), upon introducing the mutS171::Tn10Dcm insertion.

<sup>b</sup> Chromosomal DNA (1 to 5 μg), mildly sheared by vortexing for 1 to 2 min, was used for transformation (carried out as described for Table 1). Selection was to prototrophy (Leu<sup>+</sup>). Data are the averages of three independent determinations.

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### TABLE 3. Mapping S. typhi insertion mutations in S. typhimurium

<table>
<thead>
<tr>
<th>Original S. typhi strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relevant phenotype</th>
<th>Mud-P22 lysate scoring positive&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Map position (cs) in S. typhimurium&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Putative locus affected</th>
<th>Inferred map position (cs) in S. typhi&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TyT1009 Met&lt;sup&gt;-&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>66–70</td>
<td>metC</td>
<td>93–97</td>
</tr>
<tr>
<td>TyT1015 Arg&lt;sup&gt;-&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>64–65</td>
<td>argA</td>
<td>90–1</td>
</tr>
<tr>
<td>TyT1020 Ile&lt;sup&gt;-&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>85–86</td>
<td>lv operon</td>
<td>88–90</td>
</tr>
<tr>
<td>TyT1031 Leu&lt;sup&gt;-&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>1–3</td>
<td>leu operon</td>
<td>58–60</td>
</tr>
<tr>
<td>TyT1041 Phe&lt;sup&gt;-&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>56–59</td>
<td>pheA</td>
<td>4–7</td>
</tr>
<tr>
<td>NN19</td>
<td>chlor&lt;sup&gt;+&lt;/sup&gt;A</td>
<td></td>
<td>17–19</td>
<td>mod&lt;sup&gt;C&lt;/sup&gt;</td>
<td>17–19</td>
</tr>
<tr>
<td>JJ3</td>
<td>chlor&lt;sup&gt;+&lt;/sup&gt;A</td>
<td></td>
<td>17–19</td>
<td>mooA&lt;sup&gt;+&lt;/sup&gt;</td>
<td>17–19</td>
</tr>
<tr>
<td>DD46</td>
<td>chlor&lt;sup&gt;+&lt;/sup&gt;A</td>
<td></td>
<td>67–69</td>
<td>uxaC</td>
<td>95–97</td>
</tr>
</tbody>
</table>

<sup>a</sup> Strains are derivatives of S. typhi Ty2. They belong to a collection of MudJ insertion mutants obtained following the protocol of Hughes and Roth (10). Chromosomal DNA from these strains was prepared according to a previously described method (1) and was used to transform cells of strain MA5383 (metE<sup>A22</sup> metE<sup>E551</sup> galE<sup>496</sup> rpsL<sup>120</sup> sly104 Δ[Fes1] H1-b H2-ε,n x nml hsdL6 hsdS29 recD543::Tn10Dcm, selecting for Kan<sup>+</sup>). Insertions were then transferred to strain LT2 by P22-mediated transduction and were mapped, with prototrophy scored as previously described (2). For MudJ insertions conferring phenotypes other than auxotrophies, a Tet<sup>+</sup> marker was introduced within the MudJ element. This was done by using a P22 lysate prepared from a strain carrying lacZ<sup>+</sup> Tn10 to transduce the different MudJ-carrying strains, selecting for Tet<sup>+</sup>. Lac<sup>+</sup> Tet<sup>+</sup> transductants were purified and the Tn10 element was mapped on Tet<sup>+</sup> selection medium as previously described (2, 15).

<sup>b</sup> Mud-P22 lysates, enriched for selected regions of the S. typhimurium chromosome, were prepared from a collection of 54 lysogenic strains and were used for transduction as previously described (21). Typically, overlapping lysates would concomitantly score positive in the assay (Fig. 1). Only the lysate producing the strongest signal in each transduction series is shown here.

<sup>c</sup> Centisomes (cs) are from edition VIII of the S. typhimurium genetic map (19). Intervals are those of the two strongest phage signals observed.

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Selection for Kan \textsuperscript{R} yields recombinants in which the \textit{S. typhimurium} strain carrying the MudJ insertion to be mapped (for example, the \textit{S. typhi} insertions which confer chlorate resistance (affecting bacterial work, we successfully used this method for moving the MudJ od, this is not its only possible application. In a separate line of stand strains can partially circumvent the problem resulting in an auxotrophy and preliminarily for those resulting in an auxiliary analysis (data not shown). The expected positions of the various loci in the \textit{S. typhi} chromosome were obtained upon correcting for the known discontinuities between the \textit{S. typhimurium} and \textit{S. typhi} physical maps (11).

In conclusion, the data presented here show that electro-
transformation techniques combined with the use of appropriate host strains can partially circumvent the problem resulting from the lack of a suitable transduction system in \textit{S. typhi}. Although here we used genetic mapping as a test of the method, this is not its only possible application. In a separate line of work, we successfully used this method for moving the MudJ insertions which confer chlorate resistance (affecting bacterial replication in epithelial cells) from the mutagenized background in which they were originally isolated into a "fresh" \textit{S. typhi} background (3). Such backcrosses were crucial for un-
ambiguously correlating individual mutations with their respective phenotypes. Surprisingly, in these \textit{S. typhi-S. typhi} exchanges, rec\textit{D} mutational inactivation was no longer a pre-
requisite for the recovery of transformants. Similar findings were recently made with \textit{E. coli} and were ascribed to a transient inhibition of Exo V following electroschock conditions (6).

We are indebted to Nello Bossi for discussions and comments on the manuscript and J. R. Roth for strain SLA213 and strain TT1608, which carries the mupS171::Tn\text{10}D\text{cm} allele used here. We also thank reviewers for constructive criticism.

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REFERENCES


22. Zahrt, T. C., G. C. Mora, and S. Maloy. 1994. Inactivation of mismatch repair overcomes the barrier to transduction between \textit{Salmo-