Deletion Map of the Chloramphenicol Resistance Region of R1 and R100-1

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Recombination between single-site and multisite chloramphenicol-sensitive mutants of the F-like R factors R1 and R100-1 indicates that the chloramphenicol resistance region is a single structural gene coding for the 20,000-molecular weight subunit of chloramphenicol acetyltransferase.

R factor-determined chloramphenicol resistance is mediated by chloramphenicol acetyltransferase (CAT), a tetrameric enzyme of molecular weight 80,000 that inactivates the drug by 3-O-acetylation (9, 10). All F-like R factors so far tested produce the same class of CAT (class I, reference 4), except those described by Nagai and Mitsuhashi (6) which confer impermeability to the drug.

Chloramphenicol-sensitive (chl) mutants of the F-like R factors R1 and R100-1 were isolated by the penicillin screening method after mutagenesis with ethyl methane sulfonate (2). Only 6 out of 31 mutants were single-site by the criterion of reversion to wild type; the remainder behaved as multisite mutants, suggesting that many R factor deletions arise spontaneously.

The reverting mutants were further tested for cross-reacting material (CRM) with antiserum prepared against purified CAT from a wild-type F-like R factor (22, reference 3) and for residual CAT activity (Table 1). Three reverting mutants were CRM+ and the other three showed a faint precipitin band. Those mutants also had some CAT activity, but not the same three that were CRM+ (chl-30 was CAT+ CRM−, whereas chl-14 was CAT− CRM+). The defect resulting from the mutations is not known; either tetramer formation or the active site could be affected.

All possible pairwise combinations of mutants were tested for recombination in the Rec+ host Escherichia coli J5-3. Log-phase donor cultures were mixed with stationary-phase recipients to remove the surface exclusion barrier (1). After incubation for 2 h, 0.1 ml of the mating mixture was spread on agar containing chloramphenicol (20 μg/ml) for resistant recombinant colonies. A frequency of recombination as low as 1 per 106 hetero-R cells can be detected by this method. Three types of chl mutant were recognized: those with a wide spectrum of recombination that had previously been identified as point mutants (except chl-24, which is presumably a small deletion not resolved by these experiments), mutants with a limited range of recombination that presumably contained small deletions, and mutants that did not recombine with any others and which appeared to have deletions of the whole region. The lesions were ordered in a deletion map (Fig. 1).

The order of three mutations included here had previously been shown to be chl-1-2-9 by two-factor crosses (5). This order was confirmed by deletion mapping. Also, although chl-10 and chl-14 are assigned to the same position on the map, they are different mutations since they gave rise to different phenotypic properties (Table 1) and since chl-14, but not chl-10, complements chl-1. More deletions extending into that area of the plasmid are needed to resolve them further.

All pairs of mutants that recombined in E. coli J5-3 were transferred into a recA strain (JC2926) and tested for complementation. Recombination between F-like R factors does not occur in a recA host (2), allowing the study of complementation. The only factors that complemented each other were chl-1/chl-7 and chl-1/chl-14; the cells carrying these pairs of factors had a low level of resistance to chloramphenicol, and the CAT had a low specific activity, altered Km and stability (3; unpublished data), properties of interallelic complementation. No complementation restoring wild-type activity was detected. Thus, all mutants are affected in a single gene, consistent
with the biochemical evidence that CAT is a tetrameric enzyme composed of identical subunits (10).

Genes on F-like plasmids have been identified and ordered by conjugational and transdutional mapping (11) and by studies on the structures of heteroduplexes (7, 8). We describe here the fine structure of a single gene on one such plasmid.

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LITERATURE CITED


