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NOTES

Allele Replacement in *Escherichia coli* by Use of a Selectable Marker for Resistance to Spectinomycin: Replacement of the *lexA* Gene

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We replaced the *Escherichia coli lexA* gene by a segment of DNA coding for resistance to spectinomycin and streptomycin. The use of this segment expands the range of selectable markers usable for allele replacement. The availability of this null *lexA* mutation will facilitate genetic analysis of *lexA* and the SOS regulon.

The *Escherichia coli* LexA protein controls the expression of the SOS regulon (11, 17). Many genetic studies with this system involve the use of null alleles of *lexA*. The null allele of current choice is a Tn*5* insertion at the start of *lexA* called *lexA71*::Tn*5* (6), which confers a dominant selectable marker and completely eliminates LexA function. However, the use of this allele entails several technical problems. Tn*5* transposes frequently during transductions. In addition, many other useful genetic markers are also linked to Tn*5*; in particular, strains lacking LexA function must also carry a *sulA* mutation, and the most convenient available selection for this mutation is for a *sulA* allele with a Tn*5* insertion. Finally, the *lexA* DNA sequences themselves are still present in the *lexA71*::Tn*5* allele, and our detailed genetic analysis of the *lexA* gene carried on a plasmid has been greatly complicated by homologous recombination with these sequences.

In order to overcome these problems, we have substituted another selectable marker, resistance to spectinomycin (Spc^r), for the *lexA* gene and have introduced it into the chromosome by homologous recombination (18). The new allele of *lexA*, which we term *lexA300*(Del) [where "(Del)" extends the nomenclature of reference 11 and signifies a deletion], was made from plasmid pJWL197 (Fig. 1A), in which a DNA segment coding for resistance to spectinomycin and streptomycin was flanked by homology with *E. coli* sequences lying on both sides of *lexA*. Plasmid DNA was linearized by digestion with *Sac*I and *Pst*I and introduced into strain KP520 (a *sulA100*::Tn*5* derivative [3] of strain JC9387 *recB21 recC22 sbcB15* [16]) as described previously (18), and then selection for resistance to 30 μg of spectinomycin per ml was carried out. Since this level of drug gave heterogenous colony morphology, 20 μg/ml was used in all subsequent selections. Because strain KP520 and most other strains used in work on the SOS system carry an *rpsL* allele conferring resistance to streptomycin (Str^r), simultaneous selection for Str^r was not possible. In a later step, the *lexA300* marker was transduced into other recipient strains by P1 transduction and selection for Spc^r.

Several lines of evidence indicated that *lexA300*(Del) completely replaces the resident *lexA* sequences (Fig. 1B). First, recipients carrying *lexA71*::Tn*5* became sensitive to kanamycin upon transduction to Spc^r, indicating tight linkage between this marker and *lexA71*. Second, Southern analysis of DNA from Spc^r transductants of recipients carrying *lexA*$^+$ or *lexA71*::Tn*5* (data not shown) showed that the substitution altered the size of restriction fragments in the region spanning *lexA* in a manner consistent with the maps in Fig. 1B and that flanking regions were unaltered. Analysis of four of the initial Spc^r transformants of strain KP520 also showed that the chromosomal *lexA* sequences were deleted and that the omega fragment was present on fragments of the predicted size. Third, *lexA300* inactivated the LexA repressor function, as judged by several tests using *sulA*::*lacZ* operon fusions. Levels of β-galactosidase were identical to those observed in strains carrying *lexA71*::Tn*5*. When strain JL1478, a derivative of JL1436 (L. Lin and J. W. Little, manuscript in preparation) carrying *lexA3*(Ind$^-$) on the chromosome and expressing low levels of β-galactosidase, was transduced to Spc^r, it also displayed a constitutive level of enzyme. In addition, when a *lexA71*::Tn*5* strain and a *lexA300* derivative were transformed with a multicopy plasmid, pJWL147, carrying a *lacP-lexA*$^+$ operon fusion (8), the levels of β-galactosidase at various levels of isopropylthiogalactoside were identical in both strains (see reference 10 for the regulatory circuitry). We conclude that the lower map in Fig. 1B accurately depicts the substitution in *lexA300*(Del).

Our data indicate that the sequences flanking *lexA* for 100 base pairs upstream and 350 base pairs downstream of the gene are not essential for *E. coli* growth. The region upstream of *lexA* is an intergenic region between *lexA* and *dgk*, which is transcribed in the same direction as *lexA* (7). As shown by DNA sequence analysis (4, 12), distal to *lexA* is an open reading frame of at least 168 base pairs which is deleted in *lexA300*. In addition, a small proportion of *lexA* transcripts are known to read through this region (14).

Although our data demonstrate the feasibility of using the *aadA*$^+$ gene in allele replacements, certain mutations in *rpsE* also confer a Spc^r phenotype (1); we frequently obtained a background of spontaneous Spc^r cells in our transductions. Since the *aadA*$^+$ gene also confers Str^r, and *rpsE* mutations do not result in this phenotype, a double selection could be done in strains sensitive to streptomycin. In strains that are

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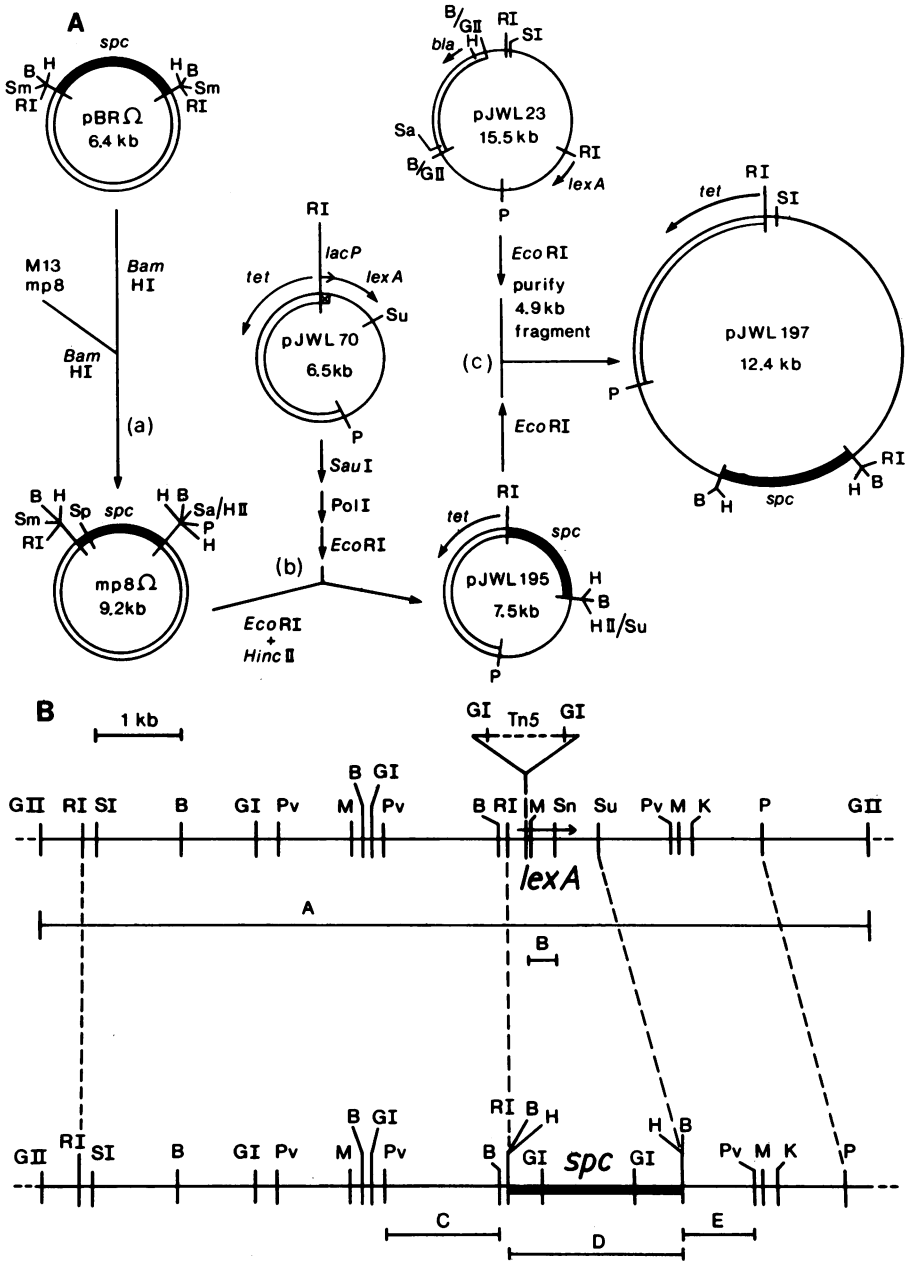

FIG. 1. Isolation and structure of *lexA300*(Del). (A) Construction of pJWL197. Vector sequences are shown by double arcs; bacterial DNA is shown by single arcs; the *lac* promoter is shown by a crossed box; and the omega fragment is shown by a filled arc. Plasmid pBRΩ, carrying the omega fragment flanked by transcription and translation terminators (15) and cloned into the *Eco*RI site of pBR322, was kindly provided by P. Prentki; bacteriophage M13 mp8 (13) and plasmids pJWL23 (9) and pJWL70 (10) were described previously. (B) Restriction maps of the *E. coli* chromosome flanking the *lexA*$^+$ and *lexA300*(Del) genes and location of probes (A through E) used in Southern analysis (not shown). Maps were derived from references 2, 5–7, and 9 and from our unpublished data. Locations of several sites outside these regions (5) are not shown but were used to interpret the Southern data. The location of Tn*5* in the *lexA71*::Tn*5* allele is shown in the upper map. B, GI, GII, H, HII, K, M, P, Pv, RI, SI, Sa, Sm, Sn, Sp, and Su, Sites for *Bam*HI, *Bgl*I, *Bgl*II, *Hin*dIII, *Hin*cII, *Kpn*I, *Mlu*I, *Pst*I, *Pvu*II, *Eco*RI, *Sac*I, *Sal*I, *Sma*I, *Sna*BI, *Sph*I, and *Sau*I, respectively. kb, Kilobase pairs.

Str^r due to *rpsL* mutations, the presence of the disruption should be confirmed by phenotypic tests or by the appropriate backcross. In the SOS system, one could demonstrate changes in LexA function or could do a backcross with a recipient such as JL1478. In general, a backcross could be done with a recipient sensitive to both drugs (although this test would be complicated by the linkage of about 70% [1] between *rpsE* and *rpsL*). Presumably, the dominance of the sensitive phenotype (1) conferred by the wild-type *rpsE*$^+$ gene could also be used.

One possible limitation to the use of the Spc^r phenotype is that, at least in our strains, selections could not be done at 5 μg of spectinomycin per ml, due to a heavy background lawn, whereas the *lexA300* allele yielded resistance only to 20 μg/ml; above this level, colony morphology was heterogeneous. Although we have not surveyed other *E. coli*

strains for levels of resistance, it may be that this window will be too small to be of use in certain backgrounds.

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