Nucleotide sequence and functional properties of DNA encoding incompatibility in the broad host-range plasmid R1162

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Summary. A 370 base pair (bp) fragment of R1162 DNA encoding the incompatibility determinant has been cloned and sequenced. The DNA is located between 6.1 and 6.5 on the R1162 map, near the origin of replication. The sequence contains three perfectly conserved 20 bp direct repeats, with 11 bp of this sequence repeated a fourth time. The direct repeat unit shows some homology with that of another, unrelated broad host-range plasmid, RK2. The cloned DNA has two other properties: it lowers the copy number of R1162 when cloned into this plasmid, and it is required in cis for replication of R1162 satellite plasmids.

Material and methods

Strains, plasmids and bacteriophage
The E. coli K-12 strains used in this study are MVI0 (thr leu thi lacY supE44 tonA AtrpE5) (Hershfield et al. 1974) and GM33 (dam-3 F-) from B. Bachmann. The latter strain was necessary to obtain undermethylated plasmid DNA suitable for digestion with BclI and MboI. The M13 derivatives mp7, mp8 and mp9 (Messing et al. 1981; Messing and Vieira 1982) were used for cloning and sequencing. The plasmids employed in this study and their derivation are listed in Table 1.

General procedures
TYE growth medium is 1% Bacto-tryptone, 0.5% Bacto-yeast extract and 0.5% NaCl. Antibiotics, added when necessary, were carbenicillin (500 μg/ml), trimethoprim (200 μg/ml), chloramphenicol (50 μg/ml), kanamycin, tetracycline and streptomycin (all 25 μg/ml). Plasmid DNA was routinely isolated by the method of either Birnboim and Doly (1979) or Holmes and Quigley (1981). For larger amounts of plasmid DNA, the procedure of Marko et al. (1982) was followed. Restriction endonucleases and T4 polynucleotide ligase were purchased from New England Biolabs, and used according to their instructions. Electrophoresis of cleaved fragments of DNA was carried out on 0.8% agarose or 5% acrylamide - 0.24% bisacrylamide gels, and the DNA visualized by staining with ethidium bromide, as previously described (Meyer et al. 1982). Cells were made competent for transformation with DNA by the method of Cohen et al. (1972).

Cloning procedures
Cloning the origin of R1162 DNA replication. The major intermediates are shown in Figs. 1 and 2. All coordinates
Because one end of the deletion is fixed at 7.4 kb, the extent of the deletion could be determined from the overall size of the plasmid derivative. We obtained by an identical method derivatives of pMS137 (Fig. 2). Here, the resulting deletions extend from 3.0 kb a variable distance toward 7.4 kb.

We transferred the R1162 DNA from the pACYC184 derivatives back into pBR322. Two plasmids were chosen for further study, pMS170 (derived from pMS105, Fig. 1), and pMS138 (from pMS137, Fig. 2). Further deletions were made of each of these, by digestion of their DNA, first partially by HpaI, and then completely by ClaI, enzymes which generate homologous cohesive termini. We screened for deletion derivatives which had lost the HindIII site in pBR322 (Figs. 1, 2). These derivatives have deletions with one endpoint fixed at the HindIII site, because the nearest HpaI site in pBR322 is in the gene for β-lactamase, and resistance to carbenicillin (Cb') was used in the selection of transformants. Thus, deletions across the ClaI site into pBR322 would not be obtained by the procedure. Two plas-