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Twelve 43-Base-Pair Repeats Map in a *cis*-Acting Region Essential for Partition of Plasmid Mini-F

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The nucleotide sequence of the DNA region involved in partitioning of plasmid mini-F has been determined. The sequence consists of 12 direct tandemly arranged repeats of 43 base pairs (the two flanking repeats, 43 plus 1 base pairs) with extensive homology to each other. Each repeat contains an additional inverted repeat of 7 base pairs.

It has been originally proposed that the stable inheritance of bacterial plasmids during cell division is controlled by a special partitioning mechanism (8) responsible for the distribution of newly replicated DNA molecules into the daughter cells. Meanwhile, functions involved in partitioning have been localized on several plasmids, e.g., P1, CloDF13, F, NR1, and R1 (1, 6, 11, 13, 14).

Plasmid mini-F consisting of the *Eco*RI f5 fragment of the F factor comprises, according to nucleotide sequence analysis, 9,569 base pairs (M. Helsberg and R. Eichenlaub, manuscript in preparation). Mini-F carries all functions essential for regulation of plasmid replication, incompatibility, and partition (11). To avoid segregation, a low-copy-number plasmid (one to two copies per chromosome) like mini-F has to operate a rather precisely acting partitioning mechanism. Indeed, a discrete DNA region has been determined to be involved in plasmid partitioning and also to be responsible for incompatibility among the IncFI group of plasmids (Fig. 1) (2, 3, 5, 9, 16, 17).

This region encodes two proteins, designated A (44 kilodaltons) and B (36 kilodaltons) (20), and a *cis*-acting region, *incD* (16) (Fig. 1). The determination of the nucleotide sequence of mini-F (Helsberg and Eichenlaub, in preparation) has shown that the direction of transcription of proteins A and B is as depicted in Fig. 1. Earlier reports on the transcription of these protein genes (21) based on R-loop analysis located the transcripts correctly but led to a misinterpretation of the direction of transcription. The A and B proteins are essential for partitioning, as demonstrated by the isolation of amber mutants (2) and by complementation experiments (16). However, the function of these two proteins remains unclear. Recently Hayakawa et al. (7) showed that B protein binds to the *incD* region. Furthermore, deletions in the *incD* region cause instability of plasmid inheritance. The level of instability seems to depend on the size and the location of the deletion (16).

To get more information on the structure of this interesting region of the mini-F plasmid, we have determined its nucleotide sequence.

The sequence was obtained by "shotgun" DNA sequencing by the method of Sanger et al. (18). Sonication of the purified *Eco*RI fragment of plasmid pHW70 (deleted coordinates 40.462F [*Bam*HI] through 47.280F [*Bgl*II]) (20) and subsequent end repair and fractionation of the DNA fragments on agarose gels were performed as reported by Deininger (4). Fragments with a size of 200 to 800 base pairs were recovered from the gel (19) and inserted into the *Hin*cII site of phage vector M13mp8 (12).

The sequence contains 12 tandemly arranged repeats of 43 base pairs (Fig. 2), located 335 to 852 base pairs from the right-hand end of the physical map of plasmid mini-F (49.869F) (Fig. 1). This DNA region contains the gene locus *incD*, also termed *sopC* (16), which was mapped in a region 380 to 740 base pairs from the rightward *Eco*RI site (5).

Four of the 12 43-base-pair repeats are completely homologous with each other, whereas the others differ in some positions from the consensus sequence (Fig. 3). This is especially pronounced in the two flanking repeats, which also have an insertion of 1 base pair. Additionally each of the repeated sequences contains a 7-base-pair inverted repeat (Fig. 3). Eight of these inverted repeats match with 100% homology, whereas two repeats contain only one base substitution. Again the inverted repeats within the flanking repeats exhibit a stronger degeneration with alterations in three and four positions, respectively. The sequence repetition results in 18 *Ava*II recognition sites in this part of the mini-F genome. Hayakawa et al. (7) reported on a spontaneous derivative of a pBR322–mini-F–A2 recombinant (mini-F fragment A2 [14], coordinates 47.629F through 49.869F) which had a deletion of about 450 base pairs removing all *Ava*II sites from the mini-F entity of the plasmid.

These authors (7) further showed that the B protein of plasmid mini-F may form a stable complex with two host-encoded proteins of 75 and 33 kilodaltons which binds to the highly repetitive *incD* region. This particular protein-DNA association may be responsible for an interaction with the membrane and expression of F1 incompatibility.

The stronger incompatibility reaction observed by Gardner et al. (5) when *incD* is cloned into a high-copy-number vector may be explained as competition of *incD* sites for the B protein-binding complex. Furthermore, the occurrence of spontaneous deletions in *incD*, as reported by Hayakawa et al. (7), may result from insufficient saturation of the *incD* region with the protein complex which further excludes the plasmid from the partitioning mechanism. In the recent publication by Hayakawa et al. (7), T. Miki et al. report in the form of a personal communication that the A2

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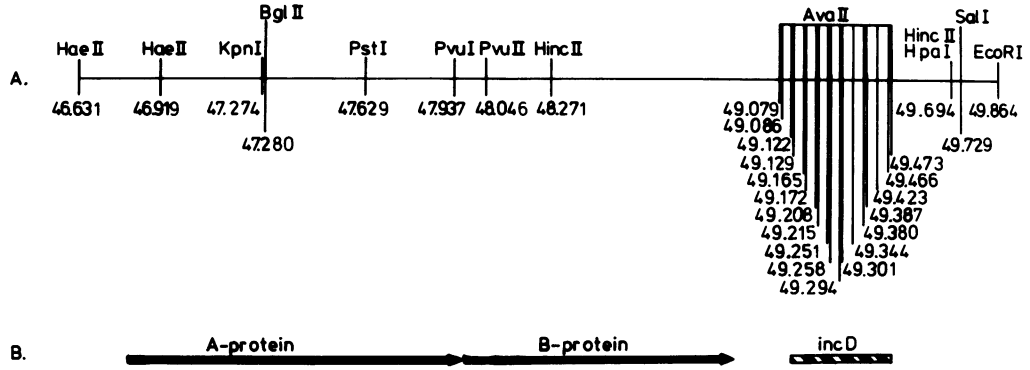

FIG. 1. Physical and functional map of the mini-F region governing partition. A, Recognition sites of relevant restriction endonucleases are shown with their corresponding F coordinates in kilobases. The coordinates for restriction sites relate to the first base pair of the recognition sequence and are corrected according to the complete nucleotide sequence of mini-F (Helsberg and Eichenlaub, in preparation), which is 9,569 base pairs long, with the two *Eco*RI sites at the borders 40.300F and 49.869F. B, Map position of functions involved in partitioning (16). Arrows indicate the putative reading frames of proteins A and B according to the nucleotide sequence (Helsberg and Eichenlaub, in preparation), also designated *sopA* and *sopB* (16) or F1 and F2 (10), respectively. The hatched box indicates the map position of the *incD* locus (5).

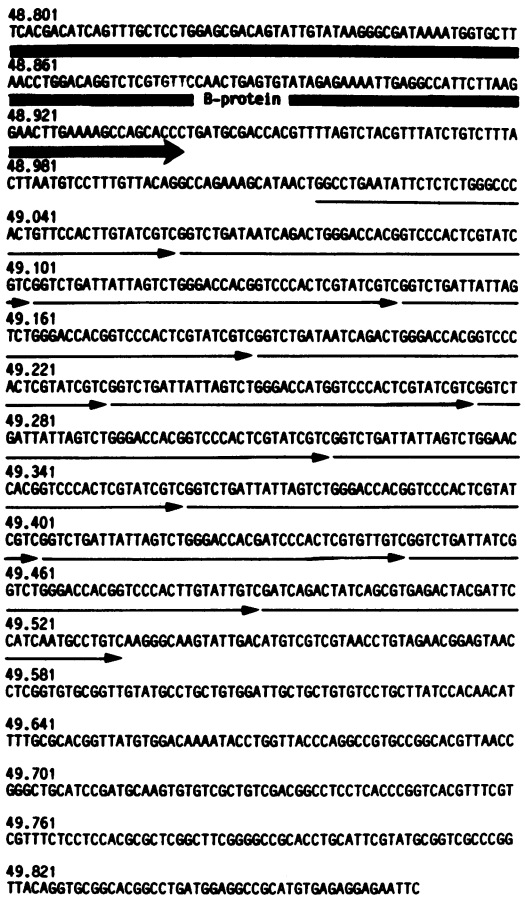

FIG. 2. Nucleotide sequence of the 12 repeating sequences. Nucleotide sequence of the mini-F region 48.801F through 49.869F. The heavy arrow marks the 3′ end of the putative coding region of the B protein. The thin arrows indicate the locations of the 12 tandem repeats.

segment of mini-F, which includes the region we have sequenced, contains at least 11 tandemly arranged, 43-base-pair repeating sequences, each of which carries an *Ava*II site.

Little is known about the role of the A protein in the partitioning mechanism. Ogura and Hiraga (16) proposed that expression of the B protein may be regulated by protein A. However, Phua et al. (17) suggested the opposite, since gene *B* mutants, obtained by transposition mutagenesis, resulted in an overproduction of protein A. The nature of the molecular interaction of all of the components involved in the regulation of partitioning functions and the mechanism of partitioning itself remain to be determined.

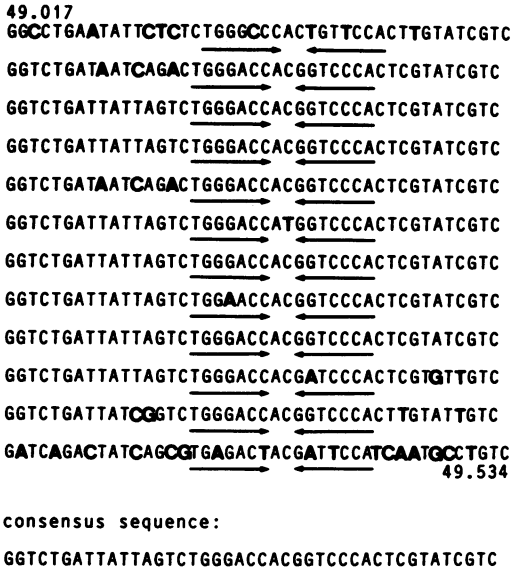

FIG. 3. Listing of the repeated sequences. Comparison of the nucleotide sequences of the 12 direct repeats. The most frequent nucleotide sequence is taken as the consensus sequence. Differences from this standard sequence are printed in bold letters. Arrows indicate the positions of the 12 internal inverted repeats.

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