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Structural Characterization of the \textit{virB} Operon of the Hairy-root-inducing Plasmid pRiA4

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The virulence \textit{(vir)} loci of the hairy-root-inducing plasmid pRiA4 are essential for pathogenicity of its host \textit{Agrobacterium rhizogenes} A4. We have now determined the nucleotide sequence of \textit{virB} region (9,926 bp). It was found that the \textit{virB} operon is composed of 11 genes (\textit{virB1} to \textit{virB11}), whose products mostly appear to be associated with the cell membrane. A novel structural characteristic is frequent overlapping between the translation termination and initiation codons of the adjacent genes. This is indicative of fine tuning of relative translation frequencies for each VirB protein, supporting the view that VirB multisubunit complexes provide facilities for T-DNA transfer at the bacterial cell membrane.

\textit{Keywords:} \textit{Agrobacterium}/ Membrane protein/ Plant-microbe interaction/ Virulence regulon

The agropine-type hairy-root-inducing plasmid pRiA4 confers tumorigenic symptoms at wound sites on a wide variety of dicotyledonous plants upon infection by its host bacterium, \textit{Agrobacterium rhizogenes} A4. Tumorigenesis is caused by the transfer of a defined DNA segment (T-DNA) on the plasmid from a bacterium into the plant nuclear genome and subsequent constitutive synthesis of plant phytohormones directed by the T-DNA. T-DNA transfer requires the 25 bp imperfect direct repeats at both extremities of T-DNA as \textit{cis} factors, and the virulence \textit{(vir)} loci outside of T-DNA as \textit{trans} factors. The \textit{vir} genes constitute the six transcriptional units, \textit{virA}, \textit{virB}, \textit{virC}, \textit{virD}, \textit{virE}, and \textit{virG}. Their expression is tightly regulated as a regulon by both the \textit{virA} and \textit{virG} gene products, being inducible by plant phenolic compounds such as acetosyringone. Nucleotide sequences of all of the \textit{vir} loci, except the largest \textit{virB} operon, have been determined by our laboratory, and compared with those of tumor-inducing plasmids harbored by \textit{A. tumefaciens} (for reviews, see ref. 1 and 2). Here, we report the nucleotide sequence of the \textit{virB} operon together with its flanking regions, thus completing sequencing of the entire \textit{vir} loci of pRiA4.

The \textit{vir} loci of pRiA4 have been identified with transposon (Tn3-HoHo1) insertion mutations that lead to defects in pathogenicity, and the \textit{vir} gene organization examined from the similarity with that of the tumor-inducing plasmid pTiA6 by hybridization experiments. As a result, it has been found that pRiA4 \textit{virB} is located in a region corresponding to the \textit{HindIII}-10, -8 and -32b frag-
ments. These three fragments were purified from a cosmid clone (pBANK1329) covering the DNA region from the HindIII-23b to HindIII-25 fragments (3), and then separately inserted into pUC18. With the resulting recombinant plasmids, progressive deletion derivatives were constructed by either ejecting appropriate restriction fragments or recloning smaller fragments. Each of the inserted DNA segments was sequenced from either side for both strands by an ABI Prism 377 DNA sequencer. In addition, adjacent restriction fragments actually being contiguous was confirmed by sequencing overlapping fragments with appropriate custom primers. By arranging overlapping sequences of both strands, the overlapping fragments with appropriate custom primers. quencer. In addition, adjacent restriction fragments actu- side for both strands by an ABI Prism 377 DNA se- the inserted DNA segments was sequenced from either section fragments or recloning smaller fragments. Each of recombinant plasmids, progressive deletion derivatives then separately inserted into pUC18. With the resulting 40.3 kDa, and 38.2 kDa. Each of the presumed coding gests that the virA contains a putative target sequence for signal peptidase for protein synthesis in all possible reading frames sug- (9,926 bp; see http://molbio.kuicr.kyoto-u.ac.jp/virb). The occurrence of initiation and termination codons for protein synthesis in all possible reading frames suggests that the virB locus carries information to code for 11 polypeptides of 26.4 kDa, 12.3 kDa, 11.7 kDa, 87.7 kDa, 23.1 kDa, 31.8 kDa, 5.9 kDa, 26.2 kDa, 32.2 kDa, 40.3 kDa, and 38.2 kDa. Each of the presumed coding frames was named virB1 to virB11, respectively, all of which were accompanied by a potential ribosome-bind- ing sequence (4). The initiation codon for all virB genes except virB1 was the conventional AUG codon. How- ever, virB1 appeared to use a rare initiation codon AUC. This was not a result of base substitution occurred during cloning experiments because direct sequencing of the corresponding region of pRiA4 DNA gave an identical result. The transcription start site is located 65 bp up- stream of the virB1 translation initiation site (5). There are helically phased vir box sequences essential for VirG binding and the promoter -10 region, though no typical promoter -35 region is present (5). These structural fea- dings on the three plasmids have evolved from a common ance- tor, at least those determining compatibility group are usually more cognate to each other than those involved in different incompatibility groups, functional domains of pRi and pTi, at least those determining virulence on plants (vir and T-DNA) and autonomous replication and incompatibility (orilim), appear to have been shuffled during evolution of these plasmids.

References