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Physical Map Location of the *Escherichia coli* Attachment Site for the P22 Prophage (*attP22*)

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The lambdoid phage P22 infects and lysogenizes *Salmonella typhimurium* (12). However, the *Escherichia coli* chromosome also contains a P22 prophage attachment site (*attP22*), and P22 can integrate into *attP22* on an *E. coli* episome carried by *S. typhimurium* (3). The *E. coli attP22* has been mapped near the proline biosynthesis genes *proBA* (3). The *E. coli proBA* and the adjacent threonine tRNA gene, *thrW*, are located at 271 to 274 kb (min 6) on the Kohara map (2, 5, 6, 9). We demonstrate here that the *E. coli attP22* overlaps *thrW* (Fig. 1).

This study was prompted by two additional lines of evidence. First, the *S. typhimurium* P22 attachment site (*ataA*) was mapped near *proBA* (4), Leong et al. (7) reported the sequence of *ataA*, and Pierson and Kahn (10) found within *ataA* a region which could be folded into a typical cloverleaf which resembled a threonine tRNA. On this basis, they proposed that, in *S. typhimurium*, P22 integrates into the 3′ end of a previously unknown gene for threonine tRNA with anticodon 5′ CGU. Second, the *E. coli thrW* (anticodon 5′ CGU) sequence contains 46 nucleotides which are identical to the P22 *ataA* core sequence in *S. typhimurium* (1, 7).

We tested for integration of P22 into the *E. coli thrW* gene present on *E. coli* episome F′128 carried in an *S. typhimurium* strain deleted for the *Salmonella proBA-thrW-ataA* region. Southern blot analysis was conducted on DNA extracted from a P22 lysogen (strain TR1825) and a nonlysogen (strain TR1810), the pair of strains used to map *attP22* genetically (3). DNA extracted from these strains was digested with *Bam*HI, *Eco*RI, or *Hin*dIII, electrophoresed, transferred to a GeneScreen membrane (DuPont-NEN Products), and probed with a 30-mer identical in sequence to the last 30 nucleotides of the *thrW* tRNA. This probe was identical also to nucleotides of the P22 attachment site core sequence. (Probing DNA from the nonlysogenic wild-type *S. typhimurium* and *E. coli* C600 cut with the same enzymes detected the fragments predicted from published restriction fragment maps [9, 11]. Probing DNA from the *Salmonella* deletion strains confirmed the deletions.)

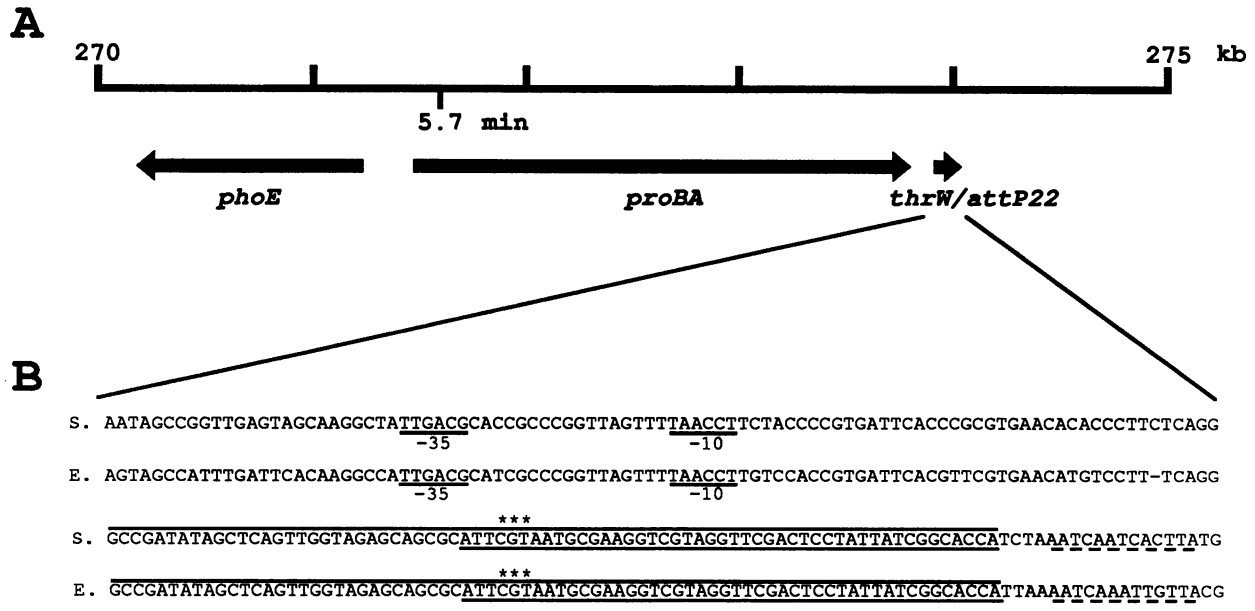

FIG. 1. (A) Physical structure of the 270- to 275-kb region of the Kohara map (5). (B) Comparison of the *E. coli thrW* gene (E) with the threonine tRNA gene-*ataA* region of *S. typhimurium* (S). Potential tRNA gene promoters are underlined and designated −35 and −10; the tRNA mature form is overlined; the *attB* core is solid underlined; a potential integration host factor site is dashed underlined. ***, anticodon.

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From the nonlysogenic, episome-containing strain TR1810 DNA, *Bam*HI, *Eco*RI, and *Hin*dIII produced hybridizing fragments of 20, 9.4, and 23 kb, respectively. In the lysogenic, episome-containing strain TR1825 DNA, the 20-kb *Bam*HI fragment (above) was replaced by two fragments of 16 and 6.6 kb, the 9.4-kb *Eco*RI fragment was replaced by two fragments of 10 and 6.0 kb, and the 23-kb *Hin*dIII fragment was replaced by two fragments of 20 and 9.0 kb. The replacement of one hybridizing fragment by two new fragments is consistent with the integration of P22 into an attachment site core, with the generation of a second *attP22* core region on the clockwise side of the prophage. The lengths of the new fragments are consistent with known sequence and restriction fragment maps, specifically the *Eco*RI and *Hin*dIII fragments from the prophage left region and *Bam*HI fragments from the prophage right region (7–9). An alternative explanation that two new fragments were generated as the result of interrupting an attachment site core tends to be ruled out because the probe was a single strand complementary to only 30 nucleotides of the 3′ end of the 46-mer core sequence.

(In addition, the probe hybridized to an additional DNA sequence from both the *E. coli* episome-containing *Salmonella* strains TR1810 and TR1825 and *E. coli* C600. This sequence, contained within 18-kb *Bam*HI, 4.8-kb *Eco*RI, and 2.4-kb *Hin*dIII fragments, shares some homology with the P22 attachment core but was not used as an attachment site.)

We thus conclude that the P22 prophage attachment site in *E. coli* is within the *thrW* gene. This work extends the recently reported 6,752-bp stretch of DNA from *pepD* through *proA* (2) to include *thrW* and *attP22* and places *thrW* and *attP22* at 274 kb on the physical map.

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