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Location of the Genes Controlling Alkaline Phosphatase on the F'13 Episome of *Escherichia coli*

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Interrupted mating experiments between F'13 and F⁻ cells showed that the location on the F'13 episome of the genes controlling alkaline phosphatase is on the end proximal to the point of entry, in the order *phoA proC phoB phoR tsx*.

The F'13 episome carried by strain W3747 of *Escherichia coli* includes the sex (F) particle and chromosomal genes corresponding to the genetic region 9 to 14 min on the *E. coli* genetic map (6, 10). This episome has originated from a single recombination event within the chromosome of an Hfr (Hfr 13) strain (Fig. 1; 9). The genes involved in the synthesis and regulation of alkaline phosphatase, *phoA*, *phoB*, *phoR* (3, 4, 12; *phoB* was previously designated as *phoT*, ref. 3; *phoT* now designates a gene involved in inorganic phosphate uptake, ref. 11), and the gene *proC* are located between *lac* (9 min) and *tsx* (10 min), and, therefore, during transfer they could be located either at the proximal or the distal end of the F'episome (see Fig. 1). An analysis of recombinants resulting from mitotic crossing-over in merodiploid strains has shown that *phoA* and *phoR* are located proximally (1). Using data obtained from interrupted mating experiments between strain W3747 and various F⁻ strains, we confirmed this finding and determined the location and order of the other genes on the F'13 episome.

Figure 2 shows an interrupted conjugation experiment between strain W3747 (*tsx*), carrying the F'13 episome, and RLA6 (F⁻ *lac proC phoR str*). All methods are described in ref. 2 and 12 and in the legends to the figures. Pro⁺ Str-r exconjugants began to appear 10 min after the mating had begun and 6 min before the Lac⁺Str-r exconjugants began to appear. The whole "length" of the F'13 episome is approximately 5 min, and the *lac* locus is located on the end distal to the point of entry (9); these results show that *proC* is located towards the end proximal to the point of entry.

The Pro⁺Str-r and Lac⁺Str-r exconjugants can be of two types. They may be F⁻ haploid recombinants resulting from episomal gene integration into the chromosome of the F⁻ parent; it is expected that during the first 16 min of the interrupted mating, before the *lac*⁺ gene and the F particle enter, all exconjugants are of this type. The second type of exconjugants are sexductants in which the entire episome, including F, has entered into the F⁻ cells, converting them into stable F' merodiploids. The frequency of the merodiploids among the exconjugants can be determined in the cross shown in Fig. 2, since these merodiploids are heterozygous for the *phoR* locus (alkaline phosphatase constitutivity). Ultraviolet irradiation of colonies of such *phoR/F'phoR*⁺ merodiploids, and spraying them 48 h later for enzyme activity, yielded sectored colonies (Fig. 3). The sectors showing alkaline phosphatase activity resulted from the formation of *phoR/F'phoR* homozygotes (3, 5). Figure 4 shows the frequency of *phoR/F'phoR*⁺ merodiploids and of Lac⁺ segregants among the Pro⁺Str-r exconjugants of the interrupted mating experiment shown in Fig. 2. The entrance of the distally located *lac*⁺ marker was simultaneous with that of F (i.e., formation of merodiploids); at 30 min they comprised over 80% of the Pro⁺ recombinants. Figure 4 also shows that among the Lac⁺Str-r

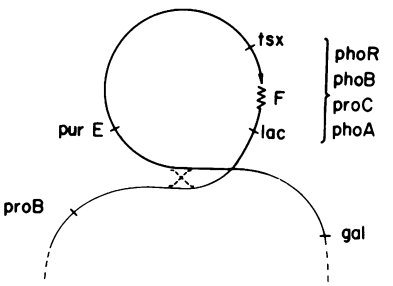
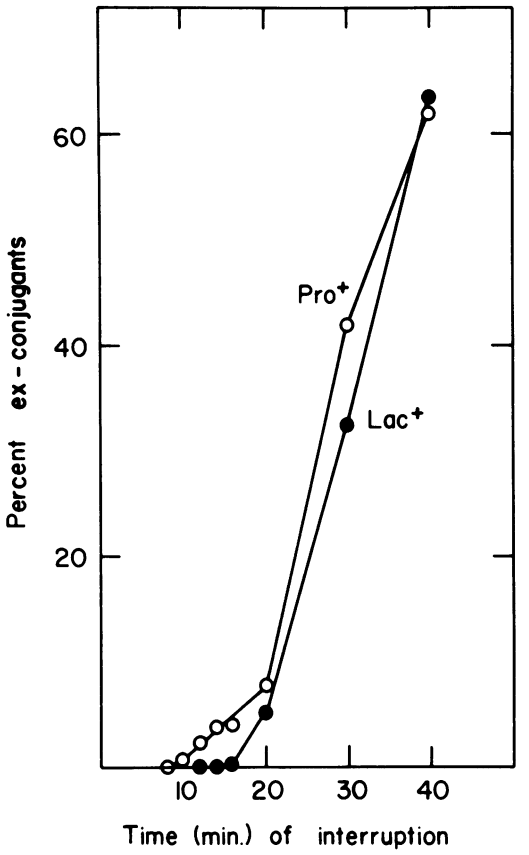


FIG. 1. *The formation of the F'13 episome in strain W3747 by a single crossover in the chromosome of strain Hfr 13 (9). The arrow represents the point of entry (origin) and the wavy line represents the sex factor (F).*



exconjugants all were Pro+ (i.e., they were sexductants). Figure 5 shows the frequency of PhoR+ and of Tsx-r haploid recombinants among the Pro+Str-r exconjugants obtained in the experiment shown in Fig. 2. Up to the 16th minute there is a sharp rise in the frequency of both types of recombinants. This rise is expected if the two markers entered after *pro*+, and it is evident that the entrance of *phoR*+ was followed by *tsx*+. The sharp decline of both markers beginning at the 16th minute was due to the increase in frequency of the merodiploid form. The genetic order of the markers on the episome is therefore *O-proC phoR tsx lac F*. Whether *phoA* (the structural gene of alkaline phosphatase) is at the proximal or the distal end of the F'13 episome was determined in the interrupted conjugation experiment between strains W3747 (*tsx*) and ALP1 (F- *lac phoA*

FIG. 2. *Transfer kinetics of proC*+ *and lac*+ *from strain W3747 (F'tsx) to RLA6 (F- lac proC phoR str). Cells were grown exponentially in broth at 37 C to approximately 5 × 10*8 *cells/ml. At time 0 they were mixed at a ratio of approximately 1F':10F-, and at intervals samples were removed, agitated for 15 s on an instrument described by Low and Wood (7), diluted, and plated on tris(hydroxymethyl)aminomethane-buffered minimal medium (4) selective for Pro*+*Str-r and for Lac*+*Str-r exconjugants. Frequency of exconjugants is expressed as percent of input of F' cells.*

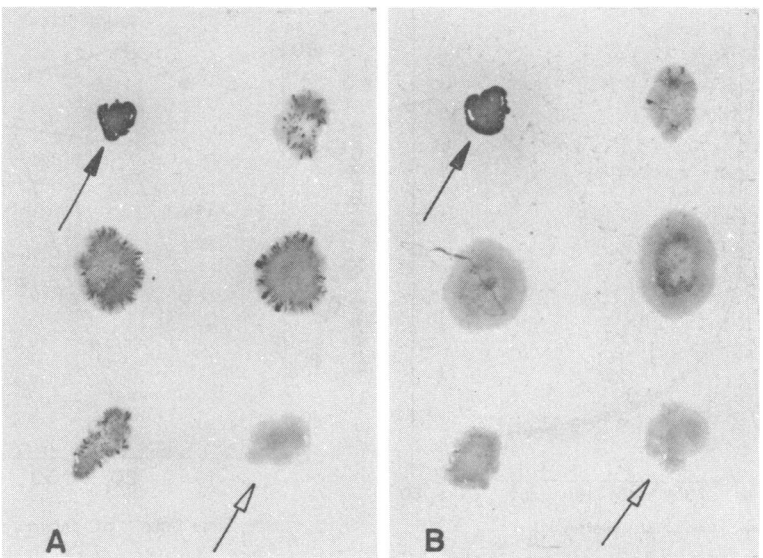


FIG. 3. *The detection of phoR/F'phoR*+ *merodiploids. Pro*+ *Str-r exconjugant colonies from the cross described in Fig. 2 were plated, using sterile tooth picks, on two plates, one of which (A) was irradiated with ultraviolet light for 30 s (approximately 10% survived) and the other (B) of which was left untreated. After 48 h at 37 C, the plates were sprayed with a mixture of α-naphthyl phosphate and Fast Blue B for alkaline phosphatase activity (2). The variagated colonies are the phoR/F'phoR*+ *merodiploids. The dark colony (dark arrows) is constitutive (PhoR-) and the light colony (light arrow) is repressible (PhoR*+*).*

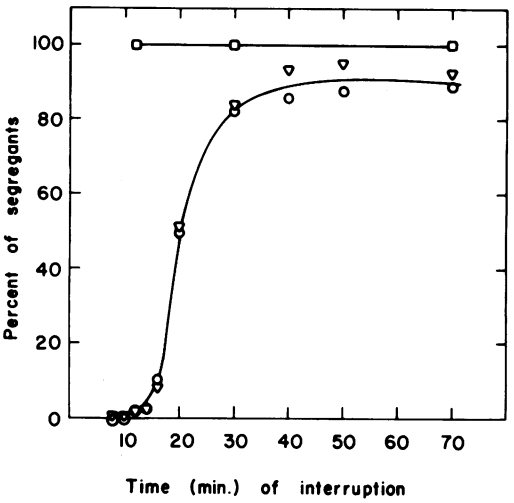


FIG. 4. *Frequency of segregants among the exconjugants formed in the cross described in Fig. 2. Symbols: O, phoR/F'phoR⁺ merodiploids among Pro⁺Str-r exconjugants; ▽, Lac⁺ segregants among Pro⁺Str-r exconjugants; □, Pro⁺ segregants among Lac⁺Str-r exconjugants.*

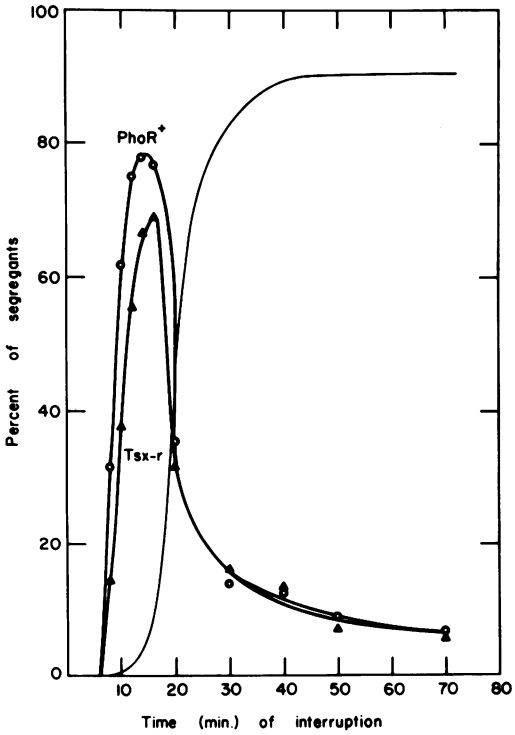


FIG. 5. *The order of phoR and tsx on the F'13 episome. Pro⁺Str-r exconjugants of the cross described in Fig. 2 were tested for phoR⁺ (O) and for tsx (Tsx-r) (Δ) segregants. The thin line represents the Lac⁺ segregants and phoR/F'phoR⁺ merodiploids shown in Fig. 4. Sensitivity to bacteriophage T6 was determined by cross-streaking (2).*

proC str). Figure 6 shows the frequency of PhoA⁺ and Tsx-r segregants among Pro⁺Lac⁻ recombinants; (we chose only Lac⁻ among the Pro⁺ to assure that we analyzed recombinants only and not sexductants). PhoA⁺ segregants appeared at a considerably higher frequency at an early time, with the appearance of Pro⁺; this shows that *phoA* is proximal, linked to *proC*, and appears before *tsx* (Fig. 6).

Finally, to determine the genetic order of all three genes in relation to *proC*, strain W3747 was crossed with three *pho* strains (Fig. 7), and the frequency of Tsx-r segregants among the Pro⁺ recombinants (group I) was compared to the frequency of Tsx-r segregants among the Pro⁺ phoX⁻ recombinants (group II, PhoX⁻ designates PhoR⁻, PhoA⁻, or PhoB⁻; see diagrams in Fig. 7). The kinetics of appearance of group I recombinants (Pro⁺Tsx-r) was similar in all three crosses. These recombinants were obtained by two genetic exchanges between the episome and chromosome. Group II recombinants (Pho⁺ PhoX⁻ Tsx-r) showed the same kinetics of appearance only with the PhoA⁻ strain (Fig. 7B), whereas with PhoR⁻ (Fig. 7A) and PhoB⁻ (Fig. 7C) the frequency of group II recombinants was much lower, indicating that more than two genetic exchanges were required (at least four exchanges; see diagrams in Fig. 7). These results are compatible with the order

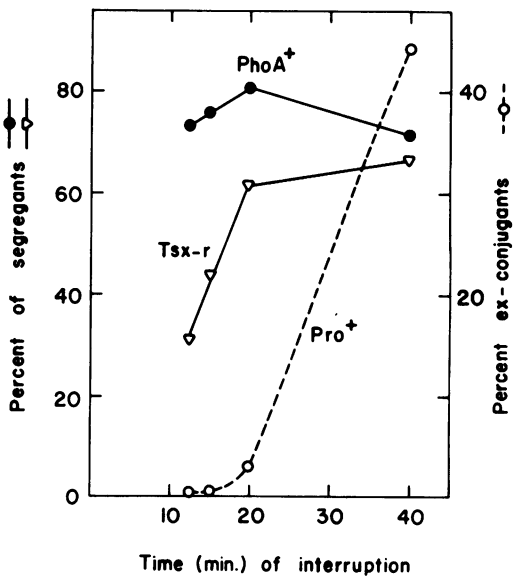


FIG. 6. *The order of phoA and tsx on the F'13 episome. Strain W3747 (F'tsx) was crossed with strain ALP1 (F⁻ lac phoA proC str) as described in the legend to Fig. 2 and plated for Pro⁺Str-r exconjugants (O). Frequency of phoA⁺ segregants (●) and of tsx (Tsx-r) segregants (▽) among Pro⁺ Lac⁻Str-r exconjugants is shown.*

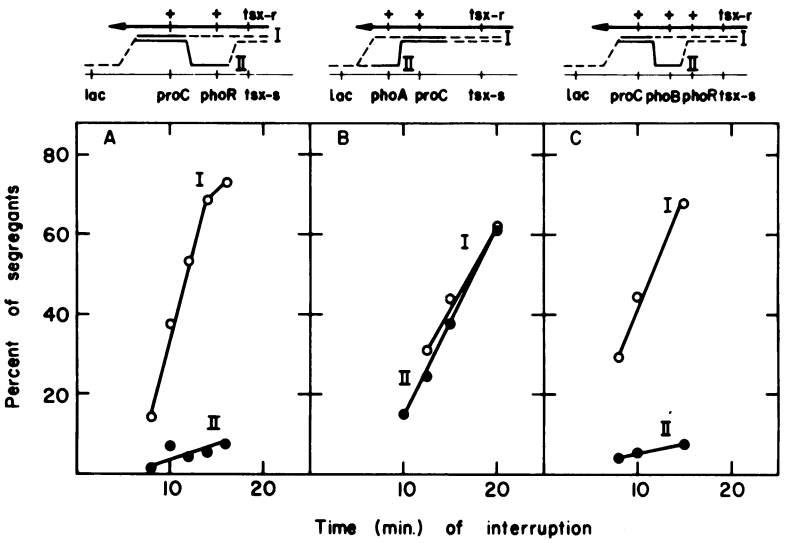


FIG. 7. *Order of phoR phoA and phoB in relation to proC. (A) W3747 (F'tsx) × RLA6 (F⁻ lac proC phoR str); (B) W3747 (F'tsx) × ALP1 (F⁻ lac proC phoA str); (C) W3747 (F'tsx) × RLP1 (F⁻ lac proC phoB phoR str). Curve I: frequency of Tsx-r segregants among Pro⁺Str-r recombinants; curve II: frequency of Tsx-r segregants among Pro⁺PhoR⁻ (A), among Pro⁺ PhoA⁻ (B), and among Pro⁺ PhoB⁻ (C) recombinants. The genotype of each parental strain is depicted in the diagrams over each figure. The arrow indicates the F' strain (W3747). Lines I and II in the diagrams show the proposed crossovers forming the corresponding phenotype. The solid part of the lines show the selected markers (proC⁺ in line I and proC⁺ phoX⁻ in line II; see text).*

O-phoA proC (phoB phoR) tsx. In cross C of Fig. 7, 5% of the Pro⁺ recombinants were constitutive (PhoR⁻), all of which were Tsx-s. This clearly shows that the gene order is *O-phoA proC phoB phoR tsx*.

In conclusion, the data reveal the following gene order: *O-phoA proC phoB phoR tsx lac F*; the *pho* genes are located towards the proximal end of the episome, and *phoA* and *phoB* (two genes which, when mutated, abolish alkaline phosphatase activity; ref. 3) are each located on another side of *proC*. The same genetic order was found on the *E. coli* chromosome (3, 8, 12).

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