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GENETIC ANALYSIS OF THE O ANTIGENS IN *SALMONELLA* IV. INHERITANCE OF O ANTIGENS 1, 3, 19 AND 34 OF *SALMONELLA* GROUP E

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The previous work (Kishi and Iseki 1973c) elucidated that the prophage attachment site of ε15 of *Salmonella* group E was near *met* and not far away from *his* on the bacterial chromosome. On the other hand, the recent reports showed prophage ε34 to be attached to or integrated into the host chromosome at a site close to the *pro* locus (Kishi 1971a, 1971b) or *pur* E (Matsuyama and Uetake 1972). The present paper deals with the genetic determinants of O antigens 1, 3, 19 of group E_4 and the prophage attachment site of ε34 on the chromosome.

MATERIALS AND METHODS

Bacterial strains. Group B strains: SW 1391 *Salmonella abony* (1, 4, 5, 12) Hfr *aro*$^-$ *met*$^-$ *str-r*; S3K-01^r *S. paratyphi* B 8006 (4, 5, 12) F$^-$ *his*$^-$ *str-r*; *i*S3K-01^r (1, 4, 5, 12), *iota*-phage-lysogenized strain of S3K-01^r. Group E_4 strains: IsF′-02 *S. senftenberg* KUBOTA (1, 3, 19) F′·lac (originally from *Escherichia coli* K12 W3747) *pro*$^-$ *str-s*; IsF′-04 *S. senftenberg* KUBOTA (1, 3, 19) F′·lac *arg*$^-$ *str-s*; Is-101 *S. senftenberg* KUBOTA (1, 3, 19) F$^-$ *pro*$^-$ *his*$^-$ *str-s*.

Bacteriological techniques. Anti-19 serum was prepared from anti-*S. senftenberg* KUBOTA serum by absorbing with *S. paratyphi* A 1015 (1, 2, 12) and *S. anatum* 293 (3, 10). Preparation methods of other monofactor sera and other bacteriological techniques employed in this experiment were described previously (Kishi and Iseki 1973a, 1973b, 1973c).

RESULTS

1. *Crosses between a group B (1, 4, 5, 12) Hfr donor and group E_4 (1, 3, 19) F$^-$ recipients*

In hybridization experiment in which the donor was *S. abony* of group B and the recipient *S. senftenberg* of group E_4, 55.0% (110/200) of the *pro*$^+$ hybrids lost O-1, 19, retaining only O-3, and 45.0% (90/200) possessed O-1, 3, 19 of the recipient type. Against

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this all the *his*$^+$, *trp*$^+$, *tyr*$^+$, *arg*$^+$ or *leu*$^+$ hybrids had O-1, 3, 19 of the recipient type. Thus O antigens of the donor were not detected in any of these recombinants. These experiments indicate that the genetic determinants of O-1, 19, or at least a part of them may reside close to *pro* on the chromosome. When the above mentioned *pro*$^+$ hybrids possessing only O-3 were infected with ε15, antigenic conversion from 3 to 3, 15 took place, and infection with ε34, caused conversion of the antigen-converted strain possessing O-3, 15 from 3, 15 to (3), (15), 34.

2. *Cross between a group E_4 (1, 3, 19) F′ donor and a group B (1, 4, 5, 12) F$^-$ recipient*

When the donor was *S. senftenberg* of group E_4 and the recipient was *iota* (P22)-lysogenized *S. paratyphi* B 8006, 45.5% (182/400) of the *his*$^+$ hybrids received O-3 of the donor, and lost O-1, 4, 5, 12 of the recipient. It is subsequently considered that the genetic determinant of O-3 may be situated at O locus close to *his*. Infection with ε15, of the *his*$^+$ hybrids possessing only O-3 (E_4B) resulted in the formation of lysogenic strain E_4B (ε15) (O-3, 15), and further infection of this latter with ε34 produced lysogenic strain E_4B (ε15, ε34) (O-(3), (15), 34). E_4B (ε15, ε34) was liable to lose ε34 during the storage.

3. *Crosses between a group B (1, 4, 5, 12) Hfr donor and E_4B (ε15, ε34) ((3), (15), 34) F$^-$ recipients*

Hybridization was performed in an anti-ε34 serum-added medium, using *S. abony* of group B as the donor and E_4B (ε15, ε34) strains as the recipients. The change of O antigen from (3), (15), 34 to 3, 15 with loss of ε34 occurred in 36.3% (87/240) of the *pro*$^+$ hybrids when the recipient was $E3_8 \cdot 60 \cdot 1$ (*pro*$^-$ *nic*$^-$), and in 48.1% (50/104) when the recipient was $E3_8 \cdot 60 \cdot 42$ (*pro*$^-$ *leu*$^-$); in 13.5% (28/208) of the *nic*$^+$ hybrids when the recipient was $E3_8 \cdot 60 \cdot 1$, and in 7.7% (8/104) of the *leu*$^+$ hybrids when the recipient was $E3_8 \cdot 60 \cdot 42$ (Table 1). And these hybrids with O-3, 15 converted into strains with O-(3), (15), 34 when infected with ε34. Of *thr*$^+$, *ser*$^+$, *asp*$^+$ or *arg*$^+$ hybrids, however, 98% of them remained to have O-(3), (15), 34, and the other changed to O-3, 15. As mentioned above, E_4B (ε15, ε34) was liable to lose ε34, and when cultured under the same condition as the above cross experiment, about 1-2% of them showed antigenic change from (3), (15), 34 to 3, 15. In this case, ε34 should be considered to be lost spontaneously but not to be abolished by the hybridization. On account of this, the pro-

Table 1. O antigen analysis of the hybrids obtained from the crosses between a *Salmonella abony* (Hfr: 1, 4, 5, 12) strain and E_4B (ε15, ε34) (F$^-$: (3), (15), 34) strains

Recipient	Selected marker	Number of recombinants 3,15	(3), (15), 34
$E3_8 \cdot 60 \cdot 1$ ((3), (15), 34)	*pro*$^+$	87	153
$E3_8 \cdot 60 \cdot 1$ ((3), (15), 34)	*nic*$^+$	28	180
$E3_8 \cdot 60 \cdot 42$ ((3), (15), 34)	*pro*$^+$	50	54
$E3_8 \cdot 60 \cdot 42$ ((3), (15), 34)	*leu*$^+$	8	96

Strains $E3_8 \cdot 60 \cdot 1$ (*nic*$^-$ *pro*$^-$) and $E3_8 \cdot 60 \cdot 42$ (*leu*$^-$ *pro*$^-$) are mutants derived from E_4B (ε15, ε34). Symbols used: *leu*, leucin; *nic*, nicotinic acid; *pro*, proline.

phage attachment site of ε34 is assumed to be located in the range of *leu-pro-nic* on the chromosome. In these cross experiments, some of *his*$^+$ hybrids possessed O antigens of the donor type (O-1, 4, 12; O-1, 4, 5, 12) and some had those of the recipient type (O-3; O(3), (15), 34).

(1) Hybrids possessing O antigens of the donor type were divided into two kinds—those containing only ε34 and those containing ε15 and ε34. The former may have received the O antigen determinants of the donor, concurrently losing not only O antigens of the recipient but also prophage ε15 from the attachment site near *his* (Kishi 1970; Kishi and Iseki 1973c). The latter is considered to receive the O antigens of the donor without losing prophage ε15. Then cross experiments were attempted in an anti-ε15 and ε34 sera-added medium using the hybrids containing only ε34 and both ε15 and ε34 as the recipients and E_4B (O-3) as the donor. When the recipient had only ε34, the *his*$^+$ hybrids possessed O-3 at high frequency, and when the recipient possessed both ε15 and ε34, the incidence of O-(3), (15), 34 was higher.

(2) Those of *his*$^+$ hybrids possessing only O-3 of recipient type had only ε34. It is therefore considered that the hybrid lost ε15 in the cross experiment, and that despite the presence of ε34, O-34 failed to be manifested because of the absence of ε15. When these ε15-deficient strains were infected with ε15, their O antigens were converted from O-3 to O-(3), (15), 34, and subsequently not only ε34 but also O-3 and O-15 were considered necessary for the production of O-34.

4. *Crosses between an E_4B (ε15, ε34) ((3), (15), 34) F′ donor and E_4B (ε15) (3, 15) F$^-$ recipients*

Hybridization experiments were carried out in an anti-ε34 serum-added medium with an E_4B (ε15, ε34) donor and E_4B (ε15) recipients. O-34 was found in 13.1% (40/305) of the *pro*$^+$ hybrids, in 6.1% (12/196) of the *nic*$^+$ hybrids, and 5.7% (10/174) of the *leu*$^+$ hybrids. In contrast with this, none of *trp*$^+$, *asp*$^+$, *cystine*$^+$ or *thr*$^+$ hybrids had O-34. The results suggest that the prophage attachment site of ε34 (*att ε34*) may be in the region of *leu-pro-nic*.

DISCUSSION

The results of reciprocal cross experiments between *Salmonella* group B strains (O-1, 4, 5, 12) and group E_4 strains (O-1, 3, 19) indicated that the genetic determinant of O-3 of *S. senftenberg* (O-1, 3, 19) is situated at the O locus near *his* on the chromosome and it is allelic to the genetic determinant of O-4, 12, and that the determinant of O-1, 19, or at least a part of it, is located at the locus close to *pro* (Fig. 1). O-1, 19 is known to show a form variation (Kauffmann 1969), and its genetic determinant, like *oaf*^{12}R of $O\text{-}12_2$ (Mäkelä and Stocker 1969), may possibly control the grade of form variation as well as the appearance or disappearance of O-1, 19. The *his*$^+$ hybrid E_4B with O-3 between a strain of group E_4 (O-1, 3, 19) donor and a strain of group B (O-1, 4, 5, 12) recipient converted its O-antigen from O-3 to O-3, 15 when lysogenized with ε15. Further infection of this E_4B (ε15) strain with ε34 resulted in another antigen converted strain E_4B (ε15, ε34) with O-(3), (15), 34.

Hybridization experiments between group B strains, E_4B (ε15) and E_4B (ε15, ε34)

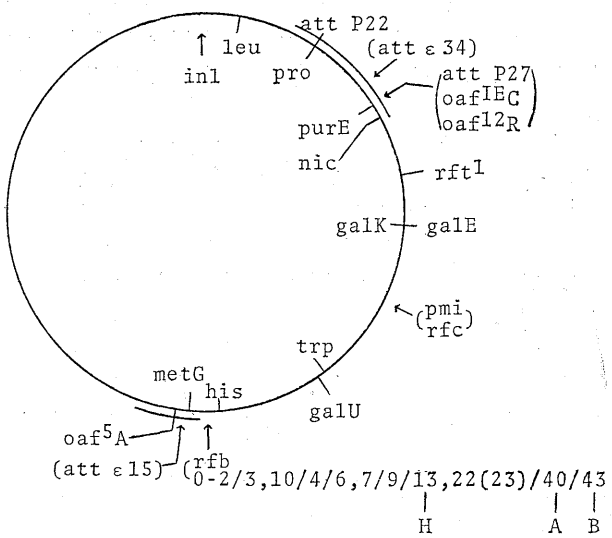

Fig. 1. Linkage map of *Salmonella*, based on the map of *S. typhimurium* (Mäkelä and Stocker 1969). Distances approximately to scale. Loci whose order is not known are bracketed. Genes named outside circle affect polysaccharide synthesis. Gene symbols: *att*-prophage attachment site for phages P22, P27, ε15 and ε34; *gal*-galactose metabolism *galE* for UDP-galactose epimerase, *galK* for galactose kinase, *galU* for UDP-glucose pyrophosphorylase I; *his*-histidine biosynthesis; *inl*-inositol fermentation; *leu*-leucine biosynthesis; *met*-methionine biosynthesis; *nic*-nicotinic acid biosynthesis; O antigen factors determined by modification of repeating unit (the factor concerned is indicated by an optinal superscript: $oaf^{5}A$ for factor 5, $oaf^{IE}C$ for factor 1 in group E, $oaf^{12}R$ for factor 12_2); *pmi*-phosphomannoisomerase; *pro*-proline biosynthesis; *pur E*-purine biosynthesis; *rf*.-LPS biosynthesis (*rfa*, of core; *rfb* (*O·2/3, 10/4/6, 7/9/13, 22(23)/40/43*) of O-repeating unit; *rfc*, polymerization of repeating unit; rft^{1}, T1 side-chain synthesis); *trp*-tryptophan biosynthesis.

indicated that the prophage attachment site of ε34 (*att* ε34) is located in the region of *leu-pro-nic* on the chromosome (Fig. 1). Since the hybridization was carried out in an anti-ε34 phage serum-added medium and the hybrid was isolated from this medium, the presence of ε34 in the hybrid is considered to have resulted from the hybridization and not to be a lysogenized strain infected with free ε34 phage in the medium.

E_4B (ε34) (O-3) is a *his*$^+$ hybrid between a group B (O-1, 4, 5, 12) donor and an E_4B (ε15, ε34) recipient. It possesses ε34 but not ε15, and manifests O antigen 3 but not O-34. When it was infected with ε15, O-15 and O-34 were produced concurrently. This fact agrees with the observation that when *S. anatum*, having ε34 and O-3, 10 but not O-34, was infected with ε15, both O-15 and O-34 were simultaneously manifested (Uetake and Hagiwara 1961). Two kinds of *his*$^+$ hybrids possessing donor type antigens (O-1,

4, 12; O-1, 4, 5, 12) were obtained from the same hybridization experiment. One possessed only ε34 while the other contained both ε15 and ε34. When O-3 was transferred from E_4B into these two kinds of *his*$^+$ hybrids, some of the former acquired O-3, while some of the latter came to possess O-(3), (15), 34. These results suggest that O-15 is manifested in the coexistence of O-3 and ε15 and O-34 in the presence of O-3, O-15 and ε34. As other similar examples are known the facts that O-4 is necessary for the manifestation of O-5 of group B (Mäkelä 1965; Kishi and Iseki 1973b), and O-12 for O-1 of groups A, B and D (Kishi and Iseki 1973a). It has thus been clarified that even when the genetic determinants of O-5 and O-1 are present they may remain as silent loci without manifesting their genetic characters unless the basal antigenic structures are in existence.

SUMMARY

The genetic determinant of O-3 of *Salmonella senftenberg* (O-1, 3, 19) is considered to be sitiuated at *O* locus near *his* on the chromosome and that of O-1, 19 close to *pro*.

The prophage attachment site of ε34 is assumed to be located in the region of *leu-pro-nic* on the chromosome.

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