Reprint from GENETICS, Vol. 46, No. 11, November, 1961. *Printed in U.S.A.*

ANOMALOUS HOMOLOGY OF FLAGELLAR PHASES IN SALMONELLA[1]

TETSUO IINO

National Institute of Genetics, Misima, Japan

Received May 30, 1961

PHASE-1 and phase-2 antigen types of a diphasic salmonella strain are determined by distinct genes, H_1 and H_2, respectively (LEDERBERG and EDWARDS 1953; LEDERBERG and IINO 1956). They are nonallelic and are transduced independently. Therefore, an H-antigen type recombinant obtained by a transduction usually expresses the antigen type of the donor in one phase, but retains that of the recipient in the other. The present paper deals with a genetic analysis of an abnormal recombinant type which expresses phase-1 types of both donor and recipient.

MATERIALS AND METHODS

Salmonella typhimurium SW1061, the original strain of the phase-1 duplicate type reported in the present paper, is an H_1-inactive mutant derived from TM2 (STOCKER, ZINDER and LEDERBERG 1953). This wild-type strain expresses *i* antigen in phase-1 and *1.2* in phase-2 (designated by *i:1.2*). SW1061 does not produce flagella in phase-1, though it carries the *i*-antigen type determinant (designated by *0(i):1.2*). The genotype of SW1061 is symbolized by Fla_1^+-$Ah_1^-H_1^iH_2^{1.2}$ (IINO 1961a). Genetic markers of the remaining salmonella strains, *S. abony* CDC-103 *b:enx* and *S. heidelberg* SL28 *0(r:1.2)*, used in the present experiment are $Fla_1^+Ah_1^+H_1^bH_2^{enx}$ and $Fla_1^-Ah_1^+H_1^rH_2^{1.2}$, respectively (LEDERBERG and IINO 1956). Fla_1, Ah_1 and H_1 are closely linked with each other and are transduced simultaneously in high frequency.

The general procedures of cultivation, transduction and selection of serotypic recombinants were conducted according to the methods of LEDERBERG and IINO (1956). Phage PLT22 was used exclusively as the mediator in transductions.

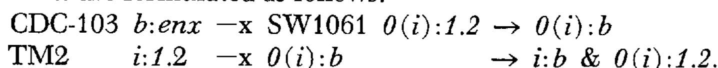
Anti-H sera were prepared by the method of EDWARDS and EWING (1955). Antigen type was determined qualitatively by slide agglutination test with antisera of titer 100, and quantitatively by tube agglutination test.

RESULTS AND CONCLUSIONS

In a transduction experiment from CDC-103 to SW1061 (IINO 1961a), a transductional clone which alternates phases between *b* and *0* (flagellaless) was

[1] Contribution from the National Institute of Genetics, Japan. No. 389. This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A.

obtained (Table 1). The mixture of a broth culture of the *0:b* clone with the lysate of a phase-2 culture of TM2 was brushed on an NGA plate supplemented with anti-*b* serum as a selective agent. Twenty swarms which developed as a result of H-transductions were isolated and typed for their antigen and alternative phase. Seven clones among them were agglutinated by anti-*i* serum, and after anti-*i* serum selection produced the alternative phase which is agglutinated by anti-*b* serum. These *i:b* type clones do not dissociate into motile and non-motile type any more. The remaining 13 clones were all agglutinated by anti-*1.2* serum. An alternative H phase has not been obtained from these clones by anti-*1.2* serum selection, but the dissociation of *0* type was observed in NGA plates. These results are formulated as follows:



CDC-103 *b:enx* —x SW1061 *0(i):1.2* → *0(i):b*
TM2 *i:1.2* —x *0(i):b* → *i:b* & *0(i):1.2*.

Thus the abnormal type of recombinant obtained in the first transduction has lost the phase-2 antigen of the recipient and expresses the phase-1 antigen type of both donor and recipient alternatively. The phase-1 antigen acquired from the donor was not replaced by phase-1 antigen but phase-2 antigen in the second step transduction.

The identity of *b* antigens of the *i:b* type and the donor strain, CDC-103, was examined by cross agglutination and absorption experiments. Antisera were prepared against 0.5 percent formalin-saline suspensions of CDC-103 and the *i:b* clone. Anti-CDC-103 serum and anti-*i:b* serum were absorbed by *i:enx* cells obtained from *S. typhimurium* TM2 *i:1.2* —x CDC-103 *b:enx*, and anti-*b* (CDC-103) and anti-*b* (*i:b*) sera were obtained, respectively. The agglutination titers of anti-*b* (CDC-103) serum against cultures of both CDC-103 and *i:b* types were identical (24,000). After complete absorption of anti-*b* (CDC-103) serum by the *i:b* cultures, the serum did not agglutinate the cells of CDC-103 any more. In parallel, anti-*b* (*i:b*) serum showed the same titer (26,000) against both CDC-103 and the *i:b* type cells, and it was completely absorbed by the cells of CDC-103. Thus, as far as the agglutination reaction is concerned, the *i:b* type has identical *b* antigen as CDC-103.

In order to test the linkage of *i* and *b* determinants of the *i:b* type, a phage PLT22 lysate of an *i:b* clone culture was mixed with a broth culture of SL28 and brushed on NGA plates. The swarms grown as a result of Fla_{I}^{+} transduc-

TABLE 1

Transduction from a diphasic strain of S. abony, *CDC-103* b:enx *to SW1061* 0(i):1.2. *Transductional clones were screened on NGA plates supplemented with anti-*1.2 *serum*

Antigen type phase-1 : phase-2	Number of transductional clones in Expt. 1	Expt. 2	Number of reversional clones: control for expt. 1
b:1.2	95	23	0
0:enx	84	13	0
i:1.2	4	1	1
0:b	0	1	0

tion were isolated and their antigen types were examined. Twenty-nine among 39 of those transductional clones were *r:1.2*. The remaining ten clones were *i:1.2* type, which was expected from the simultaneous transduction of Fla_1 and H_1. These two were the sole recombinant types, and no antigen type which contains *b* was obtained (LEDERBERG and IINO 1956, ¶ 3.5). From these results it is concluded that the antigen-*b* determinant of the *i:b* clone is not transduced linked to Fla_1 and H_1^i.

Transduction was next carried out from a *b*-phase culture of the *i:b* type clone to an *a*-phase (phase-1) culture of a diphasic strain SW925 *a:enx*. Antigen type recombinants were screened on NGA plates supplemented with anti-*a* and anti-*enx* sera. Among 44 recombinant clones, 37 expressed *i* and *enx* in phase-1 and phase-2, respectively. The remaining seven clones expressed *a* and *b*-antigen types alternatively. No recombinant showing *1.2* type was detected. The frequency of the alternative expression of *a* or *b*-antigen types is the same as that of phase variation in SW925. Thus, the *b* determinant was transduced and replaced with H_2 of the recipient.

In conclusion, the *b* determinant of the *i:b* type clone behaves exclusively as an allelic locus to H_2. The possibility that a hidden $H_2^{1.2}$ is present in the phase-1 antigen duplicate type is excluded by the antiserum selection and the transduction tests.

DISCUSSION

The possibilities of an H_1-duplication or a heterogenote of H_1 in the phase-1 duplicate strain reported in this paper are excluded for the following reasons: (1) allelism between H_2 and the *b*-type determinant and (2) the absence of $H_2^{1.2}$ in the strain. The phenomena are best explained by the assumption that the duplication of phase-1 antigen occurred by an unequal recombination resulting in the replacement of $H_2^{1.2}$ by H_1^b. The new type now behaves as if it were H_1^i H_2^b.

The occurrence of such an unequal recombination indicates a structural homology between H_1 and H_2. H_1 and H_2 might have a certain degree of synaptic affinity. The chance of synapsis of H_1 and H_2 in transduction of these genes may be very rare, as the phase-1 duplicate type reported in the present paper is the only occasion of unequal recombination out of over 3,000 serotypic recombinants which have been examined in our laboratory for the various transductional analyses of salmonella H antigens. The behavior of the translocated *b*-antigen determinant as an allele of H_2 indicates that the barrier of synapsis between H_1 and H_2 are not their own structural differences, but may be the differences in other genes involved in a transductional fragment together with H_1 or H_2. The genes which have been known to be transduced simultaneously with H_1 are Fla_1 (STOCKER *et al.* 1953; LEDERBERG and IINO 1956) and a methylator of lysine in flagellin molecules (STOCKER, McDONOUGH and AMBLER 1961). The genes homologous to these H_1-linked genes are not present in the H_2 region, instead a stability controller of antigenic phases, Vh_2, is closely linked to H_2 (IINO 1961b).

The genetic homology of H_1 and H_2 leads us to a speculation on their phylo-

genic relations: one of them might have originated by duplication and translocation of the other; the structural differentiation might have occurred between then thereafter. These postulates trace the process of evolution from the primary monophasic serotype to the diphasic type. The secondary monophasic types might have originated from the latter by inactivation of either H_1 or H_2 (IINO 1961a). The occurrence of H_1 or H_2 duplication in nature is indicated from the isolation of H_1-duplicate strains (LEDERBERG 1961) or of so called triphasic strains (reviewed by KAUFFMANN 1954).

The frequency of the alternative expression of two antigen types in the phase-1 duplicate strain is the same as that in the original transductional recipient strain SW1061: the *b* determinant translocated to the H_2 locus becomes unstable and changes its activity at the frequency specific to the H_2 locus of the strain. The stability of the H_2 state is regulated by Vh_2 which is closely linked to H_2 (IINO 1961b). Consequently the present results suggest that the presence of an antigen determinant in the vicinity of Vh_2 is essential for its action on H_2.

SUMMARY

An abnormal H-antigen type recombinant which alternatively expresses phase-1 antigens of both donor and recipient was obtained from a transduction between *Salmonella typhimurium* SW1061 and *S. abony* CDC-103.

The duplicate phase-1 antigen type determinant H_1^b of the recombinant behaves as an allelic locus of phase-2 antigen type determinant H_2. The recombinant is presumed to be originated by an unequal recombination: H_2 locus is replaced by H_1 of the donor in the transduction. The phylogenic relation between H_1 and H_2 was discussed.

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to PROFESSOR J. LEDERBERG of the Genetics Department, Stanford University, California, for his interest and advice in going through this investigation.

LITERATURE CITED

EDWARDS, P. R., and W. H. EWING, 1955 *Identification of Enterobacteriaceae.* Burgess Publ. Co. Minneapolis, Minnesota.

IINO, T., 1961a Genetic analysis of O-H variation in Salmonella. Japan. J. Genetics **36:** 268–375.

1961b A stabilizer of antigenic phases in *Salmonella abortus-equi.* Genetics **46:** 1465–1469.

KAUFFMANN, F., 1954 *Enterobacteriaceae.* 2nd ed., E. Munksgaard, Copenhagen.

LEDERBERG, J., 1961 A duplication of the H_1 (flagellar antigen) locus in Salmonella. Genetics **46:** 1475–1481.

LEDERBERG, J., and P. R. EDWARDS, 1953 Serotypic recombination in Salmonella. J. Immunol. **71:** 232–240.

LEDERBERG, J., and T. IINO, 1956 Phase variation in Salmonella. Genetics **41:** 743–757.

STOCKER, B. A. D., M. W. McDONOUGH, and R. P. AMBLER, 1961 A gene determining presence or absence of N-methyl-lysine in Salmonella flagellar protein. Nature **189:** 556.

STOCKER, B. A. D., N. D. ZINDER, and J. LEDERBERG, 1953 Transduction of flagellar characters in Salmonella. J. Gen. Microbiol. **9:** 410–433.