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The Haloacetate Dehalogenase Gene *dehH2* Carried on a Transposon Residing in a Plasmid of *Moraxella* sp. B

Abstract

The plasmid pUO1 of *Moraxella* sp. B carries two haloacetate dehalogenase genes, *dehH1* and *dehH2*. The spontaneous deletion of an about 5-kb plasmid DNA containing *dehH2* was frequently observed. The nucleotide sequence analysis of the deletion region revealed the presence of 3.2-kb repeated sequences on both sides of *dehH2*. This sequence, which was flanked by 110-bp inverted repeats and contained an open reading frame that seemed to be a transposase gene, was almost identical to IS1071, which was found in chlorobenzoate catabolic transposon Tn*5271* that resides in the plasmid of *Alcaligenes* sp. As to the structure, the *dehH2* gene flanked by two IS1071 was considered to be a class I composit transposon.

Introduction

The enzymes that cleave halogen-carbon bonds are generally called dehalogenases. Many kinds of dehalogenases are known that differ in their reaction types and substrate ranges. Among them, the enzymes that catalyze hydrolytic dehalogenation of haloaliphatic compounds are more widespreading among microorganisms and have been well studied.

The haloaliphatic dehalogenases are classified into two groups; haloalkane dehalogenase and haloalkanoic acid dehalogenase. The haloalkanoic acid dehalogenases are divided into two sub-groups on the basis of their substrate ranges. One group, termed haloacetate dehalogenase (EC 3.8.1.3), acts specifically on haloacetates to yield glycolate. The other group, termed 2-haloacid dehalogenase (EC 3.8.1.2), acts on short-chain haloalkanoic acids to produce 2-hydroxy acids. The 2-haloacid dehalogenases, which are all inactive towards fluorinated compounds, are further classified into 4 groups according to their stereo-specificity as shown in Fig. 1. Most of them act specifically on L- or D-enantiomers converting to D- or L-products, respectively. A few enzymes being active towards both stereoisomers are divided into two groups by the reaction mechanism inverting or retaining the substrate configuration.

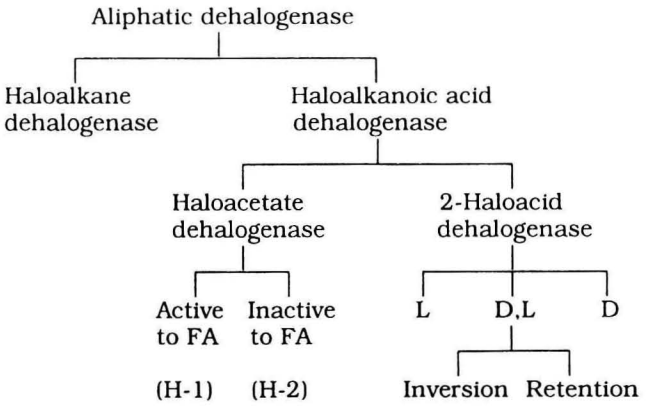

Fig. 1. Classification of hydrolytic dehalogenases acting on haloaliphatic compounds. FA indicates fluoroacetate, and L, D, and DL mean stereo-specific enzyme groups.

The group of haloacetate dehalogenase includes two types of enzymes differing in their halogen specificity. One is able to cleave the very stable fluorine carbon bond of fluoroacetate, but the other is unable. Haloacetate dehalogenases played pioneer roles in dehalogenase studies. Davies & Evans (1962) reported an enzymatic study on a haloacetate dehalogenase from *Pseudomonas dehalogenans*, being inactive towards fluoroacetate, and after that, Goldman (1965) reported a fluoroacetate-defluorinating enzyme. We also found two haloacetate dehalogenases, H-1 and H-2, which were similar with former two types of enzymes, respectively (Kawasaki *et al.*, 1981a).

In this paper, I will describe that the two types of haloacetate dehalogenases have no genetic relationship to each other. The enzyme H-1 is related to the haloalkane dehalogenase group, whereas H-2 is related to the L-stereospecific 2-haloacid dehalogenase group. It will be also reported that the H-2 gene residing in a plasmid assumes the form of a transposon.

Relationships between haloacetate dehalogenases and other dehalogenases

1. Two haloacetate dehalogenases, H-1 and H-2, are genetically unrelated.

A species of *Moraxella*, isolated from industrial waste-water, can utilize fluoroacetate and chloroacetate as sole carbon and energy souces, and has two kinds of haloacetate dehalogenases, designated H-1 and H-2, which differ in halogen specificity (Table 1). H-1 acts preferentially on fluoroacetate but has little activity against iodoacetate. H-2 acts on chloro-, bromo-, and iodoacetate, but not on fluoroacetate. Since both enzymes have only slight activity toward 2-chloropropionate, it is rational that they come under the group of haloacetate dehalogenase. The enzymes have been purified and characterized. The molecular weight estimated by SDS-PAGE is 33,000 for H-1 and 26,000 for H-2. Both

Table 1. Substrate specificity of H-1 and H-2

Substrate	Relative activity* and Km (mM) H-1	H-2
Monofluoracetate	510 (2.0)	0.0
Monochloroacetate	100 (4.8)	100 (2.5)
Monobromoacetate	70 (6.5)	160 (0.5)
Monoiodoacetate	0.2	150 (1.1)
Dichloroacetate	1.4	3.3
Trichloroacetate	0.0	0.0
2-Chloropropionate	2.6	9.5
3-Chloropropionate	<0.1	0.0
2,2-Dichloropropionate	0.0	0.1
2-Chlorobutyrate	<0.1	0.0
3-Chlorobutyrate	0.8	0.0
4-Chlorobutyrate	0.0	0.0
Chloroacetamide	<0.1	0.0

* The relative activity was expressed as percentage to the activity against monochloroacetate. Substrate concentration was 2.5 mM.

enzymes are sensitive to thiol-blocking reagents, and show similar properties as to pH and temperature (Kawasaki *et al.*, 1981b).

It was evidenced that both enzymes are specified by a plasmid named pUO1, which is about 65-kb long and transmissible into *Pseudomonas* and *E. coli.* A restriction map of this plasmid was constructed (Fig. 2) and the loci of the H-1 and H-2 genes were estimated by the cloning of the restriction fragments onto pBR322 (Kawasaki *et al.*, 1984).

When Southern hybridization was done using the *Sal*I-G fragment (Fig. 2) containing the H-2 gene as a probe, it hybridized with the *Eco*RI-F fragment containing the H-1 gene, implying that the two fragments contain common sequences. This finding and the fact that the genes for two similar enzymes were located closely on one plasmid suggested an evolutionary relationship between H-1 and H-2. However, the comparison of the H-1 and H-2 genes, termed *dehH1* and *dehH2*, respectively, in nucleotide and amino acid sequences (Fig. 3) showed no homology between two genes, implying that H-1 and H-2 must have evolved from different ancestors (Kawasaki *et al.*, 1992).

2. The H-1 enzyme is related to haloalkane dehalogenases.

Proteins and genes homologous to H-1 and H-2 and their genes were searched for in the data bases. The H-1 enzyme showed slight similarity in the amino acid sequence to a haloalkane dehalogenase (*dhlA*) of *Xanthobacter autotrophicus* GJ10 (Janssen *et al.*, 1989) and three hydrolases of *Pseudomonas putida*, i.e. 2-hydroxymuconic semialdehyde hydrolase (*dmpD*, Nordlund & Shingler, 1990), 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (*bphD*, Kimbara *et al.*, 1989), and tropin esterase (Hessing, 1983). The alignment of their amino acid sequences in relatively similar parts of N-terminal regions is shown in Fig. 4.

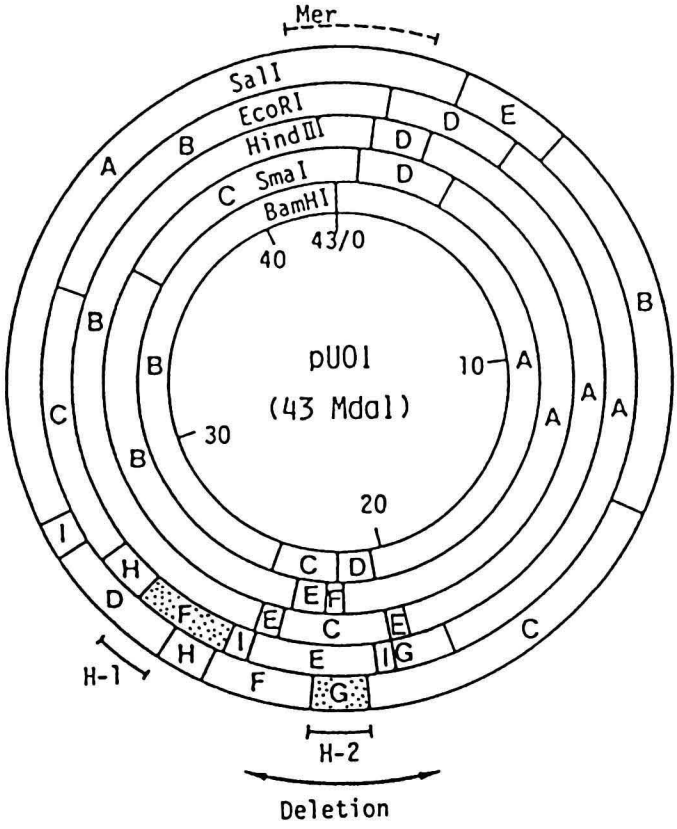

Fig. 2. A restriction map of plasmid pUO1 and the H-1 and H-2 coding regions. Dotted fragments hybridized each other.

Recently, Janssen *et al.* (1994) have reported that H-1 and two haloalkane dehalogenases encoded by the *dhlA* gene from *Xanthobacter* and the *linB* gene from *Pseudomonas paucimobilis* have a similar overall structure and catalytic mechanism.

3. The H-2 enzyme comes within L-2-haloacid dehalogenase group.

The H-2 gene showed high degrees of sequence similarity against the genes of the L-specific 2-haloacid dehalogenase group. The alignment of the amino acid sequences of seven L-2-haloacid dehalogenases and H-2 is shown in Fig. 5. H-2 shows 53% identity to DehYL from *Pseudomonas* sp. YL (Nardi-Dei *et al.*, 1994), 51% to HadL from *P. putida* AJ1 (Jones *et al.*, 1992), 51% to H-109 from *P. putida* No.109 (Kawasaki *et al.*, 1994), 50% to C-II from *Pseudomonas* sp. CBS3 (Schneider *et al.*, 1991), 44% to DhlB from *X. autotrophicus* GJ10 (Ploeg *et al.*, 1991), 38% to C-I from *Pseudomonas* sp. CBS3 (Schneider *et al.*, 1991), and 37% to Hdl IVa from *P. cepacia* MBA4 (Murdiyatmo *et al.*, 1992).

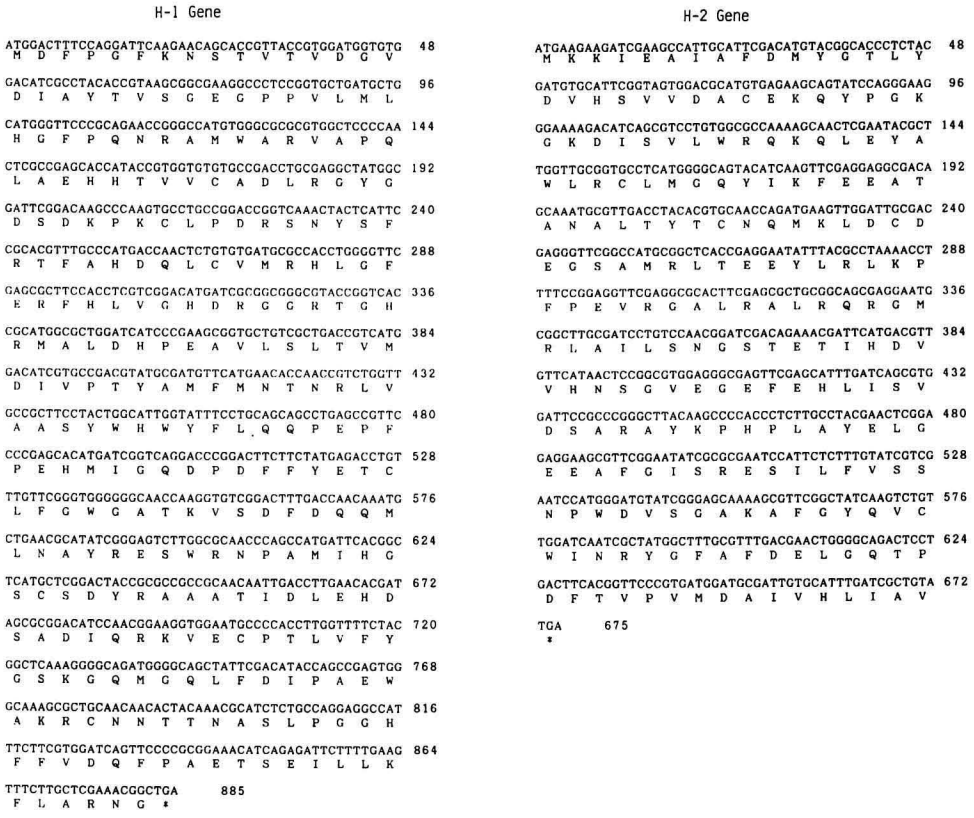

Fig. 3. The nucleotide sequences and deduced amino acid sequences of the H-1 and H-2 genes.

A tentative common sequence that was constructed by choosing the amino acid residues commonest to all 8 enzymes might be considered to be close to an original sequence, and when the 8 enzymes were compared with this sequence, H-2 showed a similarity as high as 60%, whereas other 7 dehalogenases showed 71 ~ 52% similarities. Therefore, the H-2 type haloacetate dehalogenase that has no fluorine-eliminating activity may be regarded as a variant of L-2-haloacid dehalogenase having an extremely narrow substrate range.

DehH1	23	SGEGPPVLMLHG.....	94	LGFERFHLVGHDRGG	(294 a.a.)
DmpD	26	SGAGFPLMMIHG.....	96	LEIEQADLVGNSFGG	(283 a.a.)
BphD	30	AGQGERVIMLHG.....	101	LGIEKAHLVGNSMGG	(277 a.a.)
Tpes	33	NPSGDPVLLLHG.....	99	MGLHNTTVIGHSMGS	(272 a.a.)
DhlA	44	SDAEDVFLCLHG.....	113	LDLRNITLVWQDWGG	(310 a.a.)

Fig. 4. Alignment of the predicted amino acid sequences of H-1, three hydrolases, and a haloalkane dehalogenase.

The numbers of amino acid residues in parentheses show enzyme sizes. Well conserved amino acids are emboldened. DehH1, H-1; DmpD, 2-hydroxymuconic semialdehyde hydrolase of *P. putida*; BphD, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienate hydrolase of *Pseudomonas* sp. KKS102; Tpes, tropin esterase of *P. putida*; DhlA, haloalkane dehalogenase of *X. autotrophicus* GJ10.

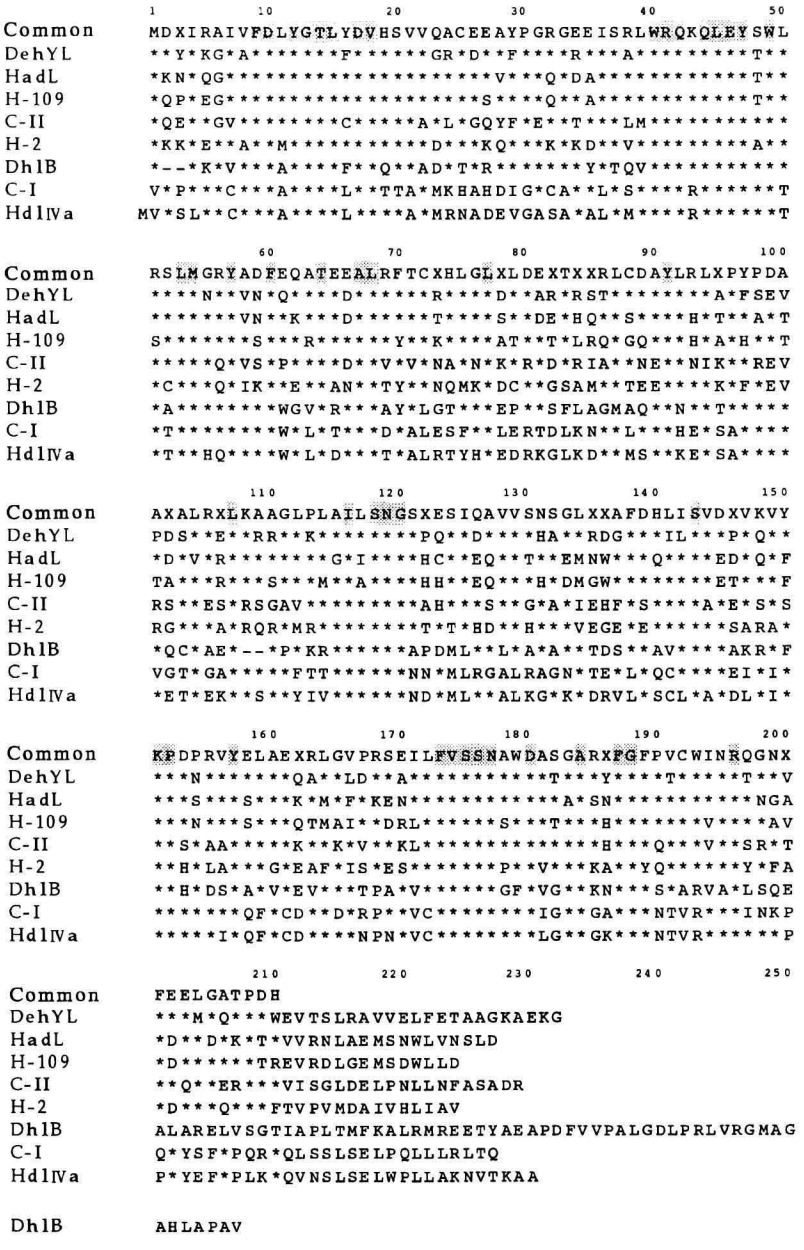

Fig. 5. Alignment of the predicted amino acid sequences of seven L-2-haloacid dehalogenases and H-2.

Asterisks indicate identical amino acids with a tentative common sequence that consists of amino acids the most common to 8 enzymes. When no common amino acid is found, X is placed. DehYL is a enzyme from *Pseudomonas* sp. YL, HadL from *P. putida* AJ1, H109 from *P. putida* No.109, C-I and C-II from *Pseudomonas* sp. CBS3, H-2 from *Moraxella* sp. B, DhlB from *X. autotrophicus* GJ10, and HdlIVa from *P. cepacia* MBA4.

A new transposon carrying the *dehH2* gene

1. The dehH2 gene is a class I composit transposon flanked by IS1071.

We knew that the phenotype H-2 of *Moraxella* was so labile that an overnight cultivation caused a few percent of the cells to lose the H-2 activity. These H-2 deficient cells harbored a uniform deletion plasmid being 5 kb smaller than pUO1. The deleted region, which was estimated by restriction analysis, included the H-2 gene and its flanking regions (Fig. 2).

The two restriction fragments, *Eco*RI-F and *Sal*I-G, of pUO1 hybridized tightly with each other as described, and besides similar restriction patterns were noticed on both sides of the deletion region. These facts suggested the presence of similar nucleotide sequences on both sides of *dehH2*. So, a plasmid DNA of about 12-kb long area including the *dehH2*-coding region and the flanking region was sequenced and analyzed.

As expected, 3.2-kb directly repeated sequences were found that holding the *dehH2* gene between (Fig. 6). Each sequence was flanked by 110-bp inverted repeats and contained single open reading frame (2913-bp) that seemed to be a transposase gene. The sequence comparison revealed that this sequence was identical, excepting three bases, to an insertion sequence element IS1071, which was first found on both ends of chlorobenzoate catabolic transposon Tn *5271* that resides in a plasmid of *Alcaligenes* sp. BR60 (Nakatsu *et al.*, 1991).

It is of interest that the same IS element was found at two distant places beyond the ocean; *Alcaligenes* strain BR60 carrying IS1071 was isolated from an industrial landfill runoff in the Niagara River watershed, USA, while *Moraxella* strain B carrying the H-2 gene was isolated in Japan.

Several transposons of class I and class II are shown in Fig. 7. The class II transposons carry short terminal inverted repeats, while class I transposons have

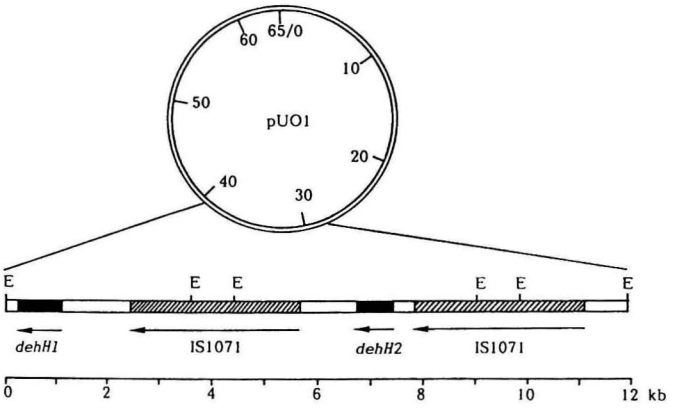

Fig. 6. Loci of *dehH1*, *dehH2* and directly repeated insertion sequences, IS1071, on plasmid pUO1.

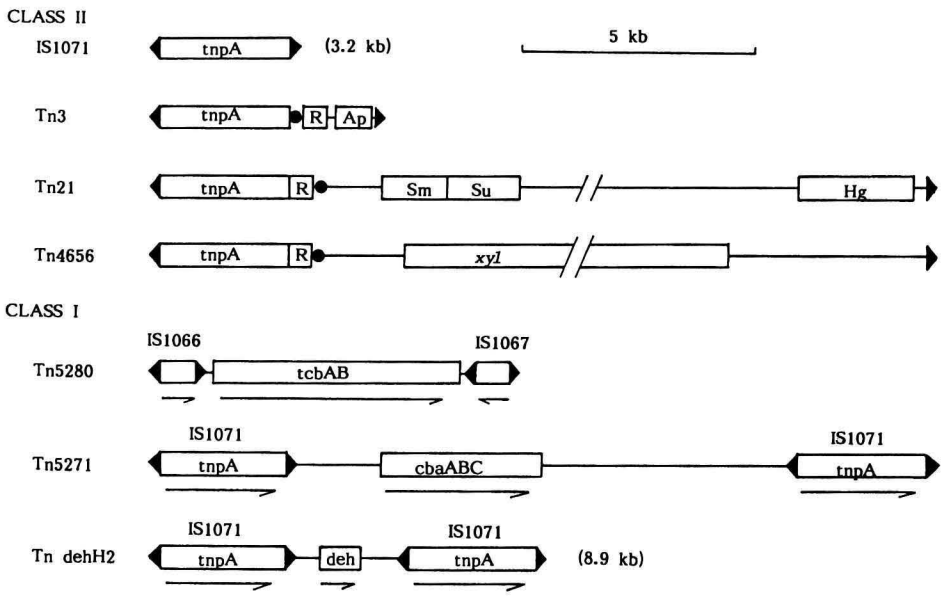

Fig. 7. Structures of class I and II transposons.

IS elements on both ends. The *dehH2* gene is sandwiched between two IS1071. Such structure supports an idea that *dehH2* is a class I composite transposon. This transposon-like element, tentatively designated Tn*dehH2*, is 8.9-kb long and consists of two 3.2-kb IS DNAs and a 2.5-kb intervening DNA containing the *dehH2* gene.

Thomas *et al.* (1992) have reported that the 2-haloacid dehalogenase gene of *Pseudomonas putida* PP3 is a transposon, designated *DEH*.

2. Functions of Tn dehH2; Deletion and transposition.

As described above, the H-2 gene is spontaneously deleted from the plasmid pUO1. The size of the deletion is corresponding to the sum of the intervening DNA (2.5 kb) and one IS1071 (3.2 kb). This deletion is seemed to be mediated by recombination between the two IS.

The transposition function of this element has been examined using the plasmid pUO1 carrying Tn*dehH2* as a donor and a drag resistant plasmid RP4 as a recipient. Two plasmids were successively introduced into the cells of *Pseudomonas* sp. E, in which pUO1 and RP4 seemed to be incompatible so that both plasmids would not coexist in the cell after several generations. Since most of the cells should have either of the two plasmids, the cells carrying recombinant plasmids could be selected by a pair of phenotypes of both plasmids, e.g. the ability to assimilate chloroacetate and kanamycin resistance. Many kinds of recombinants were obtained, and one of them, pDR4, showed suitable phenotypes, namely, the H-2 activity and all resistances (Ap, Tc, Km) and the

transmissibility owing to RP4 (Kawasaki *et al.*, 1985). Although the detailed structural analysis of recombinant DNA is in progress, I am sure that this recombinant has resulted from the transposition of this element.

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