Dehalogenase Genes of *Pseudomonas putida* PP3 on Chromosomally Located Transposable Elements

J. Howard Slater,* Andrew J. Weightman,* and Barry G. Hall†

*Department of Environmental Sciences, University of Warwick; and
†Molecular Genetics and Cell Biology, University of Connecticut

*Pseudomonas putida* PP3 utilizes halogenated alkanoic acids (HAA) such as 2,2-DCPA as its sole carbon and energy sources. Spontaneous HHA− mutants, isolated by selection for resistance to the toxic analogs monochloroacetic acid and dichloroacetic acid, arose at frequencies several orders of magnitude higher than expected for spontaneous mutations. Analysis of the five classes of mutants isolated suggested that the dehalogenase and HAA permease genes were on chromosomally located transposable elements and that the spontaneous mutations involved excision of these elements. This suggestion was confirmed by the observation that one of the elements can transpose to a target DNA molecule. The frequency of the excision event was strongly influenced by environmental conditions. Possible relationships between expression of cryptic genes and their location on transposable elements are discussed.

Introduction

*Pseudomonas putida* PP3 evolved the ability to utilize halogenated alkanoic acids (HAA) as the consequence of an event that occurred during chemostat selection with the herbicide Dalapon (2,2DCPA) as the growth substrate (Senior et al. 1976). This event, the nature of which is still unknown, led to the inducible expression of two dehalogenases and associated permeases and permitted growth on several HAAs, none of which was used by the parental strain *P. putida* PP1 (Senior et al. 1976; Slater et al. 1979; Weightman et al. 1979, 1982; Weightman 1981). Although both dehalogenase I and dehalogenase II are induced by their substrates and by a range of gratuitous inducers, the observation that they act by fundamentally different mechanisms (Weightman et al. 1982) indicates that the two dehalogenase genes are not closely related. Strain PP3 possesses no detectable plasmids (Weightman 1981; Beeching 1984); thus the genes are chromosomally located. As the consequence of the evolution of Dalapon utilization, *P. putida* PP3 became sensitive to a number of toxic, nongrowth substrates, including MCA and DCA, neither of which is inhibitory to the parent strain, PP1 (Slater et al. 1979; Weightman 1981). Thus, the organism evolved the
ability to utilize a novel resource and simultaneously became vulnerable to structurally related substrate analogs.

Although we now have a good understanding of the physiology and biochemistry of the dehalogenase system, there is at present little understanding of the evolutionary events that led to the expression of these four genes. We initiated genetic analysis of the evolution of HAA utilization by isolating spontaneous mutants resistant to the toxic analogs MCA and DCA.

**Material and Methods**

**Microorganisms**

*Pseudomonas putida* strain PP3 (2MCPA+ DCA+ MCA+ Te+ Kn+ Ap+ St+) was isolated from a microbial community growing on 2,2DCPA as the sole carbon and energy source (Senior et al. 1976) and is not related by known ancestry to other strains of *P. putida* studied in other laboratories. *Pseudomonas aeruginosa* strain PA08 (2MCPA− met− ilv− StR) containing plasmid RP4 (TcR KnR ApR) was obtained from the University of Warwick collection and was used as the source of plasmid RP4 for introduction into *P. putida* PP3. *Pseudomonas aeruginosa* strain PAO 11621 (2MCPA− leu− rmo− StR) was an StR mutant of *P. aeruginosa* PAO 1162 that was kindly provided by Professor K. N. Timmis of the University of Geneva.

**Growth and Starvation Media**

All the strains of *Pseudomonas* used in this study were grown on a liquid or solid medium containing simple mineral salts, pH 7.0 (Slater et al. 1979). 2MCPA or succinate was used as necessary as the carbon and energy sources of 0.5 g carbon liter−1 final concentration. Other compounds were added, where necessary, to give the following final concentrations: leucine, methionine, isoleucine, and valine, 25 μg ml−1; St, 500 μg ml−1; Tc, 150 μg ml−1; and Ap and Kn, 50 μg ml−1. Liquid culture growth was in 250-ml Erlenmeyer flasks incubated at 30 C on an orbital shaker at 200 rpm. For the starvation experiments, cell suspensions were incubated at 30 C in basal mineral-salts medium, pH 7.0, lacking a carbon source but containing 20 mM TCA as necessary.

For experiments involving the determination of DCAR mutants within a growing or a starving population of *P. putida* PP3, samples were removed at appropriate times and tenfold dilutions were spread-plated onto succinate mineral-salts medium to determine the total viable size of the population. DCA+ mutants were isolated on succinate mineral-salts medium containing 42 mM DCA. These plates were incubated for a standard 48 h, since prolonged incubation increased the mutant frequency (see Results).

**Mating Procedure**

Plasmid RP4 transfer was effected using a membrane-mating technique. Samples containing ~10^8 donor bacteria ml−1 and ~10^10 recipient bacteria were jointly filtered onto a sterile membrane filter (Millipore; pore size, 0.45 μm), placed on nutrient agar, and incubated overnight at 30 C. For the introduction of RP4 into *P. putida* PP3, the membranes were washed in sterile 0.02 M phosphate buffer, pH 7.0, and exconjugants were selected by plating samples (0.1 ml) onto defined medium containing 2MCPA and Tc. For the transfer of dehalogenase genes by RP4, the membranes were transferred to nutrient broth containing Tc and St and grown overnight. Exconjugants carrying dehalogenase genes and the plasmid were identified on 2MCPA mineral-salts medium supplemented with leucine and St.
Dehalogenase Separation by and Detection in PAGE

The enzyme composition of *P. putida* PP3 and the class PP4 mutants was determined using a modified method of Weightman and Slater (1980). Strains were spread-plated onto 2MCPA mineral-salts medium and incubated at 30°C for 48 h. The cells were removed by gently scraping the agar surface with a thin glass rod and resuspended in 100 μl 0.02 M Tris-SO₄ buffer, pH 7.0. Twenty-five microliters of lysozyme solution (containing 4 mg lysozyme ml⁻¹ 0.02 M Tris-SO₄, pH 7.0, buffer) was added and incubated for 5–15 min at 0°C. Seventy-five microliters of 5% (w/v) sodium EDTA was added, and the suspension was incubated for a further 15 min. Varying volumes of this cell extract (normally 70 μl) were added to PAGE wells as previously described. The gels were electrophoresed at 40 mA for ~2 h or until the marker front had traveled approximately two-thirds of the way down the gel. The gels were removed and incubated in 10% (w/v) 2MCPA solution. The sites of release of Cl⁻ ions, which were indicative of dehalogenase activity, were visualized by transferring the washed gels to a silver nitrate solution and observing the precipitation of silver chloride (Weightman and Slater 1980).

Determination of MCA Uptake

Succinate-grown closed cultures were induced during midexponential growth by addition of 10 mM of 2MCPA. After harvesting and washing, 40 ml of original culture was resuspended in ~15 ml of mineral-salts medium (Slater et al. 1979), and an aliquot was removed for protein determination. Thirteen milliliters of the suspension was pipetted into the closed reaction vessel, in which it was stirred and aerated while being maintained at 23°C. Gas released from the vessel during the experiment was bubbled through a CO₂ trap containing 8 ml of 30% (v/v) 2-aminoethanol in 2-methoxyethanol. After 15 min incubation 1 ml [¹⁴C]-MCA (1.0 μCi μmol⁻¹) was added rapidly to the suspension; this gave a final [¹⁴C]-MCA concentration of 0.5 mM. Subsequently, 1-ml samples were withdrawn from the suspension at regular intervals throughout the first 5 min of incubation in the presence of substrate. Samples were immediately passed through presoaked 0.45-μm HAWP filters (Millipore Corp.), and the filtered cells were washed twice with 5 ml mineral salts. Membranes were dried in scintillation vials overnight at room temperature. Radioactivity was determined by liquid scintillation counting.

Preparation of Plasmids and Restriction Mapping

Plasmid DNA was prepared by the method of Hansen and Olsen (1978). DNA was digested by restriction endonucleases *SmaI*, *KpnI*, *PstI*, and *XhoI* under manufacturers' suggested conditions. DNA subjected to single and all possible combinations of double digests was subjected to electrophoresis in both 0.5% and 1.0% agarose gels. Digests of bacteriophage lambda DNA were employed as size standards for the resulting fragments. A restriction map (fig. 2) was prepared by analysis of these fragments and comparison with a published map of plasmid RP4 (Lanka et al. 1983).

Results

Isolation of Mutants

Ten days after plating strain PP3, colonies were present at a frequency of 5.3 × 10⁻³ on DCA- and 3.2 × 10⁻⁴ on MCA-selective plates. All DCA<sup>R</sup> and MCA<sup>R</sup> mutants were resistant to both DCA and MCA at concentrations up to 85 mM. Two
hundred and fifty mutants were analyzed for expression of the two dehalogenases. On the basis of this analysis (table 1) four classes of mutants were identified. The PP40 class resembled the grandparental strain PP1 in that it failed to express either dehalogenase, whereas the PP42 class resembled the parental strain in that it expressed both dehalogenases. Class PP411 expressed only dehalogenase I, whereas class PP412 expressed only dehalogenase II. Some members of the PP412 class grew normally on the growth substrate 2MCPA, whereas others formed only tiny colonies on this substrate. The slow-growing strains were designated class PP4120. Growth rates were determined for representative members of each class (table 1).

Two observations suggested that permease rather than dehalogenase functions might be impaired in two classes. First, class PP42 expressed both dehalogenases and grew on 2MCPA at a rate indistinguishable from that of the parent strain PP3, yet PP42 was resistant to MCA and DCA, whereas PP3 was sensitive to them. Second, PP412 and PP4120 both expressed dehalogenase II only, yet they differed by a factor of six in terms of their growth rates on 2MCPA. Direct assays of $^{14}$C-MCA uptake (table 1) showed that all mutant classes are impaired in MCA uptake, and it appears likely that the resistance mechanism results from the reduced transport of HAAs. In support of this idea, it was previously observed that another mutant of strain PP3, strain PP309, which expressed dehalogenase I (and presumably permease activity, since the enzyme and permease are coordinately expressed) at 10 times the PP3 level, was hypersensitive to DCA inhibition (Weightman et al. 1979; Weightman 1981).

The various classes are best explained by a separate permease gene being associated with each of the two dehalogenase genes. Thus, class PP40 mutants have lost both enzymes and both permeases; PP411 mutants have lost dehalogenase II and permease II; PP412 mutants have lost dehalogenase I and permease I; PP4120 mutants have lost dehalogenase I and both permeases I and II (and depend entirely on passive

Table 1
Characteristics of Various DCA-resistant Mutants Derived from Pseudomonas putida PP3, and Comparison with P. putida Strains PP1 and PP3

<table>
<thead>
<tr>
<th>STRAIN OR MUTANT CLASS</th>
<th>GROWTH RATE ON 2MCPA (h⁻¹)ᵃ</th>
<th>RESPONSE TO DCA (85 mM)</th>
<th>DEHALOGENASE COMPLEMENT b</th>
<th>PERMEASE ACTIVITY c</th>
<th>% TOTAL MUTANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP1 . . . . . . . . . . .</td>
<td>0</td>
<td>R</td>
<td>− −</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>PP3 . . . . . . . . . . .</td>
<td>0.31 ± 0.02</td>
<td>S</td>
<td>+ +</td>
<td>8,000</td>
<td>...</td>
</tr>
<tr>
<td>PP40 . . . . . . . . . . .</td>
<td>0</td>
<td>R</td>
<td>− −</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>PP411 . . . . . . . . . . .</td>
<td>0.14 ± 0.02</td>
<td>R</td>
<td>+ −</td>
<td>1,900</td>
<td>54</td>
</tr>
<tr>
<td>PP412 . . . . . . . . . . .</td>
<td>0.23 ± 0.02</td>
<td>R</td>
<td>− +</td>
<td>2,100</td>
<td>7</td>
</tr>
<tr>
<td>PP4120 . . . . . . . . . . .</td>
<td>0.04 ± 0.01</td>
<td>R</td>
<td>− +</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>PP42 . . . . . . . . . . . .</td>
<td>0.26 ± 0.03</td>
<td>R</td>
<td>+ +</td>
<td>657</td>
<td>20</td>
</tr>
</tbody>
</table>

Note.—ND = not determined.

ᵃ Based on n = 3 independent determinations, except for PP412 (n = 4) and PP4120 (n = 6).

ᵇ Determined by rapid gel electrophoresis.

ᶜ Determined from the initial rate of uptake of ($^{14}$C)-MCA. Units are counts per minute of radioactivity take up per minute of incubation by 1 mg of cell protein.
diffusion for 2MCPA uptake, which accounts for their very slow growth rate on 2MCPA); whereas PP42 mutants have lost only permease II.

Kinetics of Appearance of Mutants

During the initial selection of mutants it was noticed that resistant colonies began to appear on selective plates after 2 days and that the number of resistant colonies appeared to increase steadily during the 10-day incubation period. In subsequent experiments, to obtain reproducible and comparable results, the number of resistant mutants on selective plates was determined after 48 h incubation. Using this criterion, we determined that the frequency of DCA\(^R\) mutants in a growing population of PP3 was \(9.0 \pm 3.0 \times 10^{-5}\), a value some 50-fold lower than that observed following 10 days of incubation on selective plates.

In subsequent experiments, when restreaked onto DCA succinate-selective plates, DCA\(^R\) mutants formed colonies in 2 days. The mutants that arose after 7–10 days incubation might have represented preexisting mutants whose growth was delayed for physiological reasons. Alternatively, they might have resulted from mutations that occurred during the selection process. If the latter were the case, the appearance of these new mutants might be simply time dependent under the nongrowth-selective conditions (DCA completely inhibits growth on succinate [Weightman et al. 1985]), or it might have been influenced by the presence of the analog DCA in the environment. These two possibilities are, of course, not mutually exclusive.

We first asked whether the frequency of mutants would remain constant in a nongrowing (carbon source-starved) population of PP3. It was found (fig. 1A) that during 9 days of starvation the frequency of DCA\(^R\) mutants rose from \(10^{-4}\) to \(10^{-2}\). The two orders of magnitude increase was not the result of differential survival rates of the mutants, since there was only a fourfold decrease in the total viable population during that time.

We next considered the influence of the analogs MCA and DCA on the rate of appearance of DCA\(^R\) mutants. Preliminary experiments showed that, in the absence of a carbon source, both MCA and DCA were highly toxic. DCA reduced the population viability by more than three orders of magnitude within 2 days, whereas MCA produced a similar reduction in 3 days. TCA, on the other hand, does not inhibit growth (J. H. Slater, unpublished observations), and population viability decreased only slightly

![Fig. 1.—Appearance of DCA\(^R\) mutants vs. time. A, Starved culture; B, culture starved in the presence of 20 mM TCA.](image-url)
faster when cells were starved in the presence of 20 mM TCA than when they were starved in the absence of TCA.

Starvation in the presence of 20 mM TCA increased the rate of formation of DCAR mutants by a factor of 100 compared with that resulting from starvation alone (fig. 1B), so that after 9 days the entire population consisted of DCAR cells. Again, this four orders of magnitude increase in DCAR mutants occurred over a period of time when there was only a tenfold decrease in population viability. In both cases the distribution of mutant classes was approximately that found in the initial survey.

A Heuristic Model

A model that explains the appearance of DCAR mutants must account for (1) the apparently enormous mutation rate, (2) the fact that the rate is influenced by environmental conditions, and (3) the fact that four out of the five classes—constituting 80% of the mutants—involves the loss of more than one function.

Simple base-pair substitutions cannot account for the observations. First, the spontaneous mutation frequency in growing populations was at least two orders of magnitude higher than is expected for spontaneous point mutations. Second, simple starvation—a condition that has not, to our knowledge, been shown to be generally mutagenic—increased that frequency another 100-fold. Third, the presence of a nontoxic analog, TCA, increased that frequency to 100%. Finally, the loss of all four functions (class PP40) occurred at about the same frequency as did the loss of a single function (class PP42); and the most common class, PP411, involving the loss of two functions, occurred 2.5 times more often than did the loss of a single function (the PP412 class). Although the genes might be organized to produce a polycistronic message, there is no order of these genes that permits the five classes to be accounted for by simple polar mutations. We have considered a variety of regulatory schemes; however, none is able to explain all five mutant classes as the consequence of single events.

The loss of multiple functions can be most satisfactorily explained by events that involve the direct loss of DNA—namely, deletions. However, this is unlikely, since the conditions employed are not known to stimulate deletion events. More important, if deletions were occurring under the conditions employed, there would be an enormous loss of viability, since these events would be expected to be randomly distributed throughout the genome. In fact, there was only a tenfold drop in viability under the conditions that led to formation of a population that consisted entirely of DCAR mutants. Clearly, the events we observed could not have arisen from randomly distributed deletions, and it therefore follows that any deletion model must explain the specificity with which the dehalogenase genes were lost.

One way that genes could be lost specifically without affecting general cell viability would be if the genes were on transposable elements that were excised during cellular stress. Since no class PP4110 (loss of dehalogenase I and both permeases) was detected, we propose that the dehalogenase genes may be organized on three Tn elements: Tn-dehA carrying dehalogenase I and permease I, Tn-dehB carrying dehalogenase II, and Tn-dehC carrying permease II. Loss of element A would produce the PP412 phenotype, and loss of element C would produce the PP42 phenotype. We believe (1) that the loss of only element B would render the cells hypersensitive to MCA and DCA as a result of the rapid accumulation of these halogenated alkanoic acids since both permeases are present and (2) that this mutant class would probably not be recovered. Excision of both elements B and C would produce the PP411 phenotype, whereas loss
of elements A and C would produce the PP4120 phenotype. Loss of all three elements would produce the PP40 phenotype.

This model does not account for the observed frequencies of various classes if excision of each element is an independent event. However, there is no reason to expect a priori that excision events would be independent, and there are some reasons to think that they may not be (see Discussion).

Testing the Model

This model leads to many predictions, only two of which are tested here. First, if the dehalogenase genes are on transposable elements, then they ought to transpose to target DNA molecules. The plasmid RP4, which carries a TcR determinant, was introduced into PP3 to produce strain PP3-R1. Strain PP3-R1 was mated with the StR Pseudomonas aeruginosa strain PAO11621. TcR StR exconjugants were selected, and those carrying dehalogenase genes were identified on 2MCPA minimal medium. In matings with unstarved PP3-R1 cells, the frequency of exconjugants bearing dehalogenase genes was $8 \times 10^{-6}$ per TcR exconjugant, whereas the frequency was $3 \times 10^{-5}$ in PP3-R1 cells that had been starved for 3 days in the presence of 20 mM TCA. No colonies appeared when cultures of PAO11621 were plated alone onto 2MCPA minimal medium. Since the plasmid RP4 does not form R-primes itself (Van Gijsegem and Toussaint 1982) and since the recipient P. aeruginosa strain cannot synthesize a dehalogenase system itself (R. C. Wyndham and J. H. Slater, unpublished observations), these frequencies reflect the frequency of transposition of the dehalogenase genes to the target plasmid. To confirm that one or more dehalogenase genes were located on plasmid RP4, four of the independently isolated putative RP4::Tn-deh plasmids were transferred to another P. aeruginosa strain by mating and selecting for TcR recipients. The 2MCPA+ phenotype cotransferred with TC+ at a frequency of 0.94.

Sixty-five RP4::Tn-deh plasmids from the mating of P. putida PP3-P1 and P. aeruginosa strain PAO11621 were selected, and every one synthesized only dehalogenase I. This is entirely consistent with the model, since only the proposed Tn-dehA encodes both a permease and a dehalogenase, dehalogenase I.

The RP4 plasmids carrying the dehalogncase I gene might have arisen by legitimate recombination between RP4 and the strain PP3 chromosome. Were that the case, all of the recombinant plasmids would contain the inserted DNA at the same location. In contrast, if the dehalogenase gene was on a transposable element, it would be expected to integrate at a variety of sites within RP4.

We first mapped one plasmid, pUU007 (fig. 2), and determined that it contained

![Restriction map of plasmid pUU007](image)

**Fig. 2.**—Restriction map of plasmid pUU007. The thick line indicates RP4 DNA; the thin line indicates insert DNA containing the dehalogenase genes. Letters indicate the sites where the DNA is cut by restriction endonucleases PstI (P), KpnI (K), SmaI (S), and XhoI (X).
a 10.2-kb insert at the 25-kb position on the standard RP4 map (Lanka et al. 1983).

We next digested plasmid RP4 and three of the RP4 derivatives carrying a dehalogenase gene with endonuclease Smal (fig. 3). All three dehalogenase plasmids (pUU001, pUU006, and pUU007) gave different digestion patterns, indicating that the inserts were into different sites. In pUU001 the insertion is into the region between 31 and 38 kb on the RP4 map, and in pUU006 it is into the region between 5 and 13 kb. In each case the dehalogenase plasmids exhibit the same 2.8-kb Smal fragment that is entirely internal to the insert. These observations indicate that the dehalogenase I gene can transpose to a variety of sites in the target plasmid RP4 and is thus unlikely to have been transferred by a classical recombination mechanism. This view is supported by the observation that the same genes can be mobilized into a variety of sites in the related plasmid R68, 45 (Beeching 1984).

A second prediction is that, if mutants arose through excision of one or more Tn elements, then the dehalogenase genes in classes PP411, PP412, PP4120, and PP42 should themselves be subject to excision. This was tested with strains PP411-004 and PP412-006 by subjecting them to starvation in the presence of 20 mM TCA. Figure

---

**Fig. 3.**—Digests of plasmid RP4 and derivatives carrying dehalogenase genes. Plasmids were digested with Smal endonuclease and subjected to electrophoresis in 0.6% agarose. Lane 1, mixture of EcoRI and HindIII fragments of bacteriophage lambda DNA; Lane 2, RP4; Lane 3, pUU001; Lane 4, pUU006; Lane 5, pUU007. The sizes in kilobases of the λ marker fragments in lane 1 are as follows (from top to bottom): 23.13, 21.23, 9.46, 7.42, 6.68, 5.80, 5.64, 4.88, 4.36, 3.53, 2.32, and 2.07.
Dehalogenase Genes of *Pseudomonas putida* PP3

4 shows that the frequency of 2MCPA^- mutants rose rapidly. These data are consistent with the model in which the two dehalogenases are on separate Tn elements.

**Discussion**

The dehalogenase genes of *Pseudomonas putida* strain PP3 exhibit the following two very unusual features: (1) Under conditions of environmental stress one or more of the gene functions is lost at extremely high rates that can result in 100% of the population having lost one of the functions, and (2) one pair of genes (dehalogenase I and permease I) can transpose as a unit to a target DNA molecule. It is not unreasonable to expect that these two unusual features are functionally related as two aspects of the same phenomenon, and we have, therefore, suggested that the dehalogenase genes are located on transposable elements that can both transpose and be excised from the chromosome.

The proposal that these catabolic genes are separately located on Tn elements in the chromosome is unusual only in that these elements seem to be natural residents of a chromosome, rather than of a plasmid or bacteriophage. Campbell (1981) suggested that some chromosomal genes in *Escherichia coli* might be especially prone to translocate and later (Campbell 1983) discussed the potential importance of transposon-mediated specific rearrangements in evolution.

We were first led to this proposal by the observation that the great majority of the spontaneous DCA^- mutants had lost more than one of the four dehalogenase functions (dehalogenase I, permease I, dehalogenase II, or permease II) and that the five classes of mutants could not be explained either by polar mutations within an operon, single deletion events, or regulatory mutations. The loss of these functions occurs at a significant rate in populations of growing cells, giving a frequency of $9 \times 10^{-5}$ DCA^- mutants in such populations, but that rate is enormously enhanced by environmental conditions. The observation that, after 10 days in the presence of the nontoxic analog TCA, 100% of the population had become DCA^- led us to coin the term “dumping” to refer to the loss of these functions. It is tempting to equate dumping with excision of these genes from the chromosome; however, we do not yet have any direct physical evidence for such loss of DNA. Whatever the physical mechanism, it is clear that dumping occurs as a direct response to environmental stress and that it occurs at the greatest rate when that stress is closely related to the function of the dehalogenase genes—i.e., in the presence of TCA. It is not difficult to understand

![Graph](image-url)
why such dumping is strongly advantageous to the cell. The permeases associated with the dehalogenases have a broad specificity and are able to transport both metabolizable substrates and the toxic analog MCA (table 1). The dehalogenase regulatory elements are also unable to distinguish substrates from toxic analogs, in that all substrates and analogs act as inducers of the system (Slater et al. 1979). Given these properties of the dehalogenase gene system, dumping may provide the only means of survival in the presence of toxic analogs.

The observation that dumping occurs does not, by itself, provide evidence that dehalogenase genes are located on transposable elements. The observation that the dehalogenase I-permease I genes can transpose to a target DNA molecule, however, demonstrates that at least these genes are on a Tn element. The failure to detect transposition of dehalogenase II or permease II is consistent with the idea that these genes are located on separate elements that would be unlikely to cotranspose to the same target DNA molecule. We know from transposons such as Tn3 (Chou et al. 1979), Tn5 (Berg and Berg 1983), and especially from studies of bacteriophage lambda that transposition can be both regulated and specific. Tn5 at least can both transpose and excise, although excision is less frequent than transposition (Berg and Berg 1983). If dumping does involve excision, it would appear that excision of the dehalogenase transposon is more frequent than transposition and that excision is subject to specific regulation (induction of dumping by TCA), whereas transposition does not appear to be dramatically stimulated by environmental conditions. Although it may be coincidental that the dehalogenase genes are located on transposons and that they can be dumped in response to specific environmental signals, it seems more likely that dumping does involve excision of these elements.

Hall et al. (1983) have recently suggested that normally silent or "cryptic" genes may play an important role in microbial evolution. They have argued that such genes would be activated under one set of environmental conditions where they would be beneficial but would be again silenced under alternative conditions where their expression would be disadvantageous. A mathematical analysis by Li (1984) supports their view. The dehalogenase genes of *P. putida* would appear to have exactly those properties suggested for cryptic genes. In the presence of substrates such as 2MCPA they are beneficial, whereas in the presence of DCA or MCA they are distinctly disadvantageous. Indeed, in the original *P. putida* strain (PP1) the dehalogenase genes were silent. All four genes were activated during selection for the utilization of 2,2DCPA (Senior et al. 1976). An important aspect of the Hall model for retention of cryptic genes is the ability of the population to repeatedly cycle between the cryptic and the expressed state of the gene. Excision of dehalogenase genes would appear to violate this aspect of the model by preventing subsequent reexpression of the genes. However, we have isolated 2MCPA+ revertants of some of the PP40 mutants isolated in this study. This observation strongly suggests that the information for dehalogenase and permease was not irrevocably lost in these mutants.

Finally, we would like to speculate that the location of dehalogenase genes on transposable elements is related to the primary event that led to expression of these genes in strain PP3. We speculate that the *deh* transposons in the original strain, PP1, are located in a region of the chromosome that prevents expression and that a replication-transposition event put a copy of the genes into a position permitting expression. Excision of these copies during DCA challenge would leave the original genes intact and available for future transposition events leading to expression.
LITERATURE CITED


MasaToshi Nei, reviewing editor

Received May 22, 1985; revision received July 16, 1985.