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Catabolic transposons

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Abstract

The structure and function of transposable elements that code for catabolic pathways involved in the biodegradation of organic compounds are reviewed. Seven of these catabolic transposons have structural features that place them in the Class I (composite) or Class II (Tn*3*-family) bacterial elements. One is a conjugative transposon. Another three have been found to have properties of transposable elements but have not been characterized sufficiently to assign to a known class. Structural features of the toluene (Tn*4651*/Tn*4653*) and naphthalene (Tn*4655*) elements that illustrate the enormous potential for acquisition, deletion and rearrangement of DNA within catabolic transposons are discussed. The recently characterized chlorobenzoate (Tn*5271*) and chlorobenzene (Tn*5280*) catabolic transposons encode different aromatic ring dioxygenases, however they both illustrate the constraints that must be overcome when recipients of catabolic transposons assemble and regulate complete metabolic pathways for environmental pollutants. The structures of the chlorobenzoate catabolic transposon Tn*5271* and the related haloacetate dehalogenase catabolic element of plasmid pUO1 are compared and a hypothesis for their formation is discussed. The structures and activities of catabolic transposons of unknown class coding for the catabolism of halogenated alkanoic acids (*DEH*) and chlorobiphenyl (Tn*4371*) are also reviewed.

Introduction

Microbiologists have long noted a common feature of bacteria that degrade environmental contaminants and those that are resistant to antibiotics. These organisms often exhibit instability in their phenotypes. This property is so pervasive that we routinely assume a standard precaution in working with these isolates in the laboratory. Whether or not there is any direct evidence of instability, we usually ensure that selection pressures are applied whenever we subculture these organisms. Implicitly we recognize the importance of maintaining selection conditions in order to maintain the organism's catabolic or resistance genotype.

Given this all-pervasive attitude, it is surprising that there are so few cases of genetic elements coding for organic pollutant biodegradation that have been characterized as true mobile genetic elements or transposons, compared to the incidence of mobile antibiotic resistance determinants. The first transposable element specifying resistance to the antibiotic ampicillin (Tn*3*) was reported in 1974 (Hedges & Jacob 1974). Rearrangements of the genes for toluene catabolism were first noted only a few years later (Bayley et al. 1977) and attributed to transposition in 1978 (Jacoby et al. 1978; Nakazawa et al. 1978; Chakrabarty et al. 1978). In the intervening years many thousands of transposable elements carrying antibiotic resistance determinants have been described (Sherratt 1989; Galas & Chandler 1989; Grinsted et al. 1990). However, in the same time period less than ten catabolic transposons specifying metabolic pathways for the degradation of organic compounds have been described, plus a few instances of catabolic genes being associated with insertion sequences (Table 1). This discrepancy may have arisen for several reasons. The selection pressure for the evolution of novel resistance mechanisms may be more severe than the pressure to evolve novel

Table 1. Catabolic transposons and other mobile catabolic elements.

Element	Class[a]	Catabolic determinants	Reference
Transposons of known class			
Tn*951*	II	Lactose	Cornelis et al. 1978
Tn*3411*	I	Citrate	Ishiguro & Sato 1984
Tn*4651*	II	Toluene	Tsuda & Iino 1987
Tn*4653*	II	[Tn*4651* – Toluene]	Tsuda & Iino 1988
Tn*4655*	II[b]	Naphthalene	Tsuda & Iino 1990
Tn*5271*	I/II[c]	Chlorobenzoate	Nakatsu et al. 1991
Tn*5280*	I	Chlorobenzene	van der Meer et al. 1991
Tn*5276*	C-Tn[d]	Sucrose	Rauch & de Vos 1992
Transposons of unknown class			
DEH	?	Halogenated alkanoates	Thomas et al. 1992
Tn*4371*	?	Chlorobiphenyl	Springael et al. 1993
[Tn*5272*][e]	?	[Tn*5271* – Chlorobenzoate]	Cashore 1993
Transposon structures of unknown mobility			
pUO1 [IS*1071*]	I/II[f]	Halogenated alkanoates	This review and Kawasaki et al. 1992
pOAD2 [IS*6100*]	I	Nylon oligomers	Kato et al. 1994

[a] Transposon classes I and II are as defined by Kleckner (1981).
[b] Tn*4655* is a Class II element defective in cointegrate formation but capable of resolution.
[c] Tn*5271* is a composite Class I element flanked by Class II insertion sequences (IS*1071*).
[d] A conjugative transposon.
[e] Tentative designation.
[f] A composite Class I structure flanked by Class II insertion sequences (IS*1071*-like). See discussion in text.

catabolic pathways in the environment. In the former case the selection pressure is negative, in the sense that antibiotics kill or inhibit growth, while in the latter case the selection pressure is positive. Resistance determinants often involve a single gene, while catabolic pathways must often be assembled from many genes and sometimes more than one operon. Therefore the evolution of resistance transposons may be a more frequent event than the assembly of catabolic transposons. Finally, we should also consider the possibility that catabolic transposons are widespread, but that we have not expended the effort required to find large numbers of different elements.

Industries devoted to the bioremediation of toxic organic pollutants are growing rapidly worldwide. As this approach to cleaning our environment gains acceptance we might expect greater effort to be made in understanding the underlying genetic, physiological and ecological characteristics of microorganisms involved in these treatment processes. It is inevitable that such basic understanding will lead to new insights into the structure, evolution and distribution of mobile genetic elements. This review has been written in the hopes of promoting an understanding of this capacity of microorganisms to restructure their genetic determinants for catabolic pathways and to donate or acquire these determinants to or from other organisms. The review will consider mobile catabolic elements of all types, whether they specify biodegradative pathways for environmental contaminants or natural products. Related reviews that also consider the properties of mobile genetic elements and catabolic genes have appeared recently (Commandeur & Parsons 1990; Sayler et al. 1990; Chaudhry & Chapalamadugu 1991; van der Meer et al. 1992; Harayama et al. 1992; Veal et al. 1992; Williams 1994).

Classical transposon structures

Bacterial transposable elements (excluding phage Mu) fall into three well defined structural classes (Kleckner 1981). Class I elements include insertion sequences (IS), containing the genetic determinants for transpo-

sition only, and composite transposons formed when flanking IS elements mobilize an intervening sequence (Syvanen 1988). IS elements exhibit a great deal of diversity at the sequence level, both in their inverted repeats and their transposase genes, so that many families of these elements can be recognized. It is possible, in many cases, to trace the distribution of similar IS elements across species and genus boundaries. The Class I catabolic transposons that have been characterized to date are listed in Table 1.

Class II elements, or the Tn*3* family of transposons, are related by inverted repeat (IR) sequence similarities, transposase and resolvase amino acid sequence similarities, and by transposition mechanism (Sherratt 1989; Grinsted et al. 1990). These elements form a more unified group than the Class I elements, although as will be discussed in this review, the known diversity within the Class II elements is growing rapidly. The class II catabolic transposons that have been characterized are listed in Table 1.

Conjugative transposons represent a third class of mobile genetic element (Clewell & Gawron-Burke 1986; Murphy 1989; Scott 1992). They all carry genes required for excision, conjugal transfer to a new host and insertion, as well as various antibiotic resistance markers. A group of nisin resistance conjugative transposons found in *Lactococcus lactis* also encode the ability to ferment sucrose and may therefore be classed as catabolic transposons (Table 1).

A number of mobile genetic elements have been described that exhibit the properties of transposable elements but that have not been characterized sufficiently to assign to Class I or II, the class of conjugative transposons, or to new classes. These are listed in Table 1 as mobile genetic elements of unknown class. Finally, sequence analyses and DNA-DNA hybridization studies have identified transposon structures for which no evidence of transposition has yet been found (Table 1).

The catabolic transposons of known class

The emphasis in this discussion of transposons of known class has been placed on their transposition determinants and on the structural features that shed light on their evolution. The catabolic pathways encoded by these elements are not discussed in detail.

Tn*951*, the lactose transposon

The 50 kb plasmid pGC1, originally isolated from *Yersinia enterocolitica*, encodes a Lac^+ phenotype that has been shown to be expressed from genes that are similar to the *lacI, lacZ* and *lacY* genes of the *E. coli* lactose operon. The genes for lactose utilization on this plasmid have been shown to be transposable as a discrete 16.6 kb element, designated Tn*951* (Fig. 1). This element has been shown to transpose into multiple sites on the broad-host-range plasmid RP1 as well as into other replicons (Cornelis et al. 1978, 1979, 1981). The sites of integration into RP1 were clustered in the region of Tn*1* (Ap^r) and between the *aph* (Km^r) and *pri* genes. It was shown that transposition of Tn*951* required the expression, in *cis* or *trans*, or a *tnpA* gene from the Tn*1*/Tn*3* family of Class II transposons. In addition, the 41 bp inverted repeats of Tn*951* were found to contain a sequence identical to the 38 bp inverted repeats of Tn*3*. This sequence similarity extends into the 3′-end of the *tnpA* gene, located adjacent to the right inverted repeat of Tn*951*, however there exists only a remnant of the *tnpA* gene in this position in Tn*951*. The reason for this became apparent when it was found that Tn*951* contains another Class II transposon, Tn*2501*, related to the Tn*21* family of Class II elements (Michiels & Cornelis 1984). Tn*2501* contains no selectable marker. It cannot complement the defective *tnpA* gene of its Tn*951* host element. The right inverted repeat of Tn*2501* ends at the exact point where the sequence of the defective *tnpA* gene of Tn*951* ends. This shows that Tn*951* evolved by insertion of Tn*2501* into an ancestral element related to Tn*3*, and that sometime during the evolution of this element most of the original Tn*3*-related transposase and resolvase genes were deleted.

Interestingly, Tn*951* contains an IS*1* insertion sequence within the element and adjacent to the *lacIZY* operon (Cornelis & Saedler 1980). Intramolecular transposition of the IS*1* element caused deletions and an inversion of Tn*951* DNA, resulting in Lac^- phenotypes. There are several examples of Class I insertion elements being found within Class II resistance transposons. For example, IS*161* (1.6 kb) is found inserted in the central region of the Tn*21*-family element Tn*2425* (Meyer et al. 1985). There is also a functional IS element (IS*904*) within the conjugative catabolic transposon Tn*5276* (Rauch et al. 1990; Rauch & De Vos 1992; see below). This combination of diverse mobile genetic elements adds to the repertoire of poten-

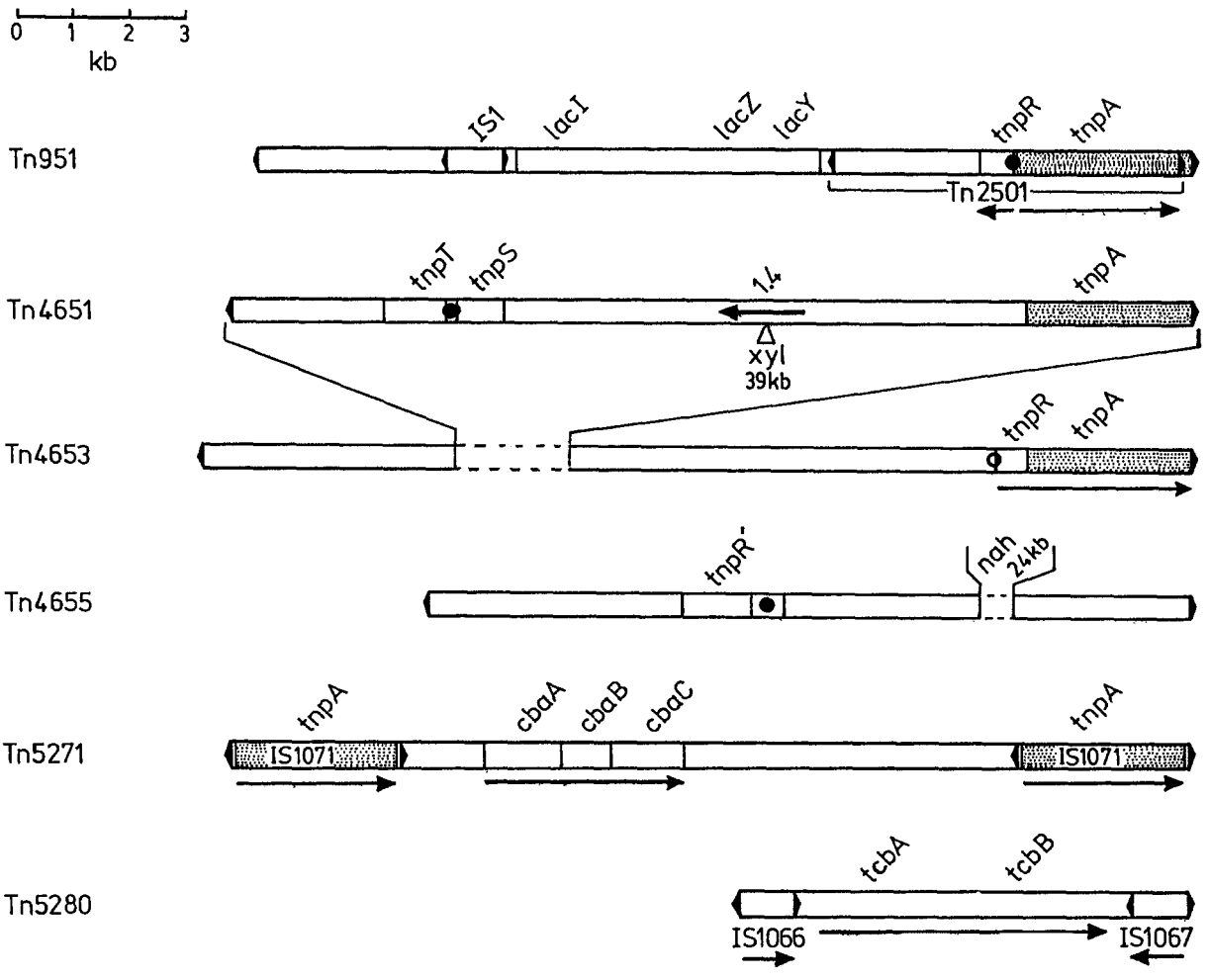

Fig. 1. Structures of the catabolic transposons of known class. Individual elements are described in detail in the text. The *tnpA* (shaded) and *tnpR, tnpR', tnpS, tnpT* genes are involved in Class II transposition and cointegrate resolution, respectively. Loci for site-specific resolution (*res*) are indicated by •. The *res* site for Tn*4653* is half-filled to indicate it is non-functional (see text). Inverted repeat sequences defining the ends of IS elements and Class II transposons are indicated by ◀. The directions of transcription of some genes, where this is known, are indicated by arrows below the transposon maps. Other abbreviations in order of appearance are: Tn*951* (lactose): IS*1*, insertion sequence; *lacI lacZ* and *lacY*, genes of the lactose operon; Tn*4651* (toluene): *tnpT* and *tnpS*, trans-acting resolution genes; 1.4, the 1.4 kb sequence involved in recombination of *xyl* genes (*xyl* genes not shown); Tn*4653* (toluene): dashed lines indicate the position of Tn*4651* nested within this transposon; Tn*4655* (naphthalene): *tnpR'*, a unique resolvase locus, distinct from *tnpR*; *nah*, the 24 kb segment encoding the naphthalene and salicylate catabolic genes (not shown); Tn*5271* (chlorobenzoate): *cbaABC*, 3-chlorobenzoate-3,4-dioxygenase and dihydrodiol dehydrogenase genes; Tn*5280* (chlorobenzene): IS*1066* and IS*1067*, insertion sequences; *tcbAB*, chlorobenzene dioxygenase and dihydrodiol dehydrogenase genes.

tial rearrangements of coding DNA that can occur within these transposons.

Tn*3411*, the citrate catabolic transposon

Genes specifying citrate utilization on the naturally occurring CIT plasmid (pOH3001) of *E. coli* have been shown to transpose as a discrete 7.4 kb fragment from this plasmid to the λ *bb* transducing phage and from the lysogenic state of this phage to pBR322 in a *recA*-deficient *E. coli* host (Ishiguro et al. 1982). Structural analysis suggested a Class I element and this was confirmed with the characterization of identical direct repeats of IS*3411* flanking the unique region of the element (Ishiguro & Sato 1984, 1988). IS*3411* is 1,309 base pairs long, with imperfect 27 base pair inverted repeats and a single open reading

frame encoding a putative 240 amino acid transposase. The distribution of this element within the Enterobacteriaceae is unknown. CIT plasmids have been found to belong to IncW, IncH1 and other incompatibility groups, suggesting either that the *cit* genes have transposed into different replicons, or that these catabolic plasmids have evolved independently on several occasions (Ishiguro et al. 1982).

Tn*5276* and Tn*5301*, the nisin-sucrose conjugative transposons

Conjugative transposons carrying genes for the catabolism of sucrose under anaerobic conditions have been described in *Lactococcus lactis* (Dodd et al. 1990; Rauch & de Vos 1992). These elements carry the *sacA* gene for sucrose-6-phosphate hydrolase, a key enzyme in the sucrose phosphotransferase pathway of sucrose fermentation. Tn*5276* is a 70 kb element which is flanked by A+T-rich termini that contain some direct repeat sequences, but without significant inverted repeat symmetry. There is evidence that conjugative transposition of this element shows some site specificity (Rauch & de Vos 1992).

Tn*4651* and Tn*4653*, the toluene transposons

Transposition of the nested toluene catabolic transposons Tn*4651* and Tn*4653* (Fig. 1) has been shown to occur by cointegrate formation and resolution, mechanisms characteristic of Class II elements (Tsuda & Iino 1987, 1988). The Tn*4651 tnpA* gene product is required for transposition of Tn*4651* and cannot complement mutations in other transposase genes, including *tnpA* mutations in the encompassing Tn*4653* transposon. The Tn*4653 tnpA* gene product, on the other hand, can complement Tn*1721 tnpA* mutations. This suggests the Tn*4653* transposase is closely related to the Tn*1721* family of Class II transposases (Grinsted et al. 1990), while the transposase of the smaller element, Tn*4651*, has diverged significantly in function and probably in amino acid sequence. Unfortunately the Tn*4651* sequence is not available for comparison to other transposase sequences.

These evolutionary considerations are supported by other features of the toluene catabolic transposons. Resolution of cointegrates formed by Tn*4651* or Tn*4653* transposition requires three factors, all of which are encoded by the smaller element, Tn*4651*. These are the *cis*-acting *res* region and *trans*-acting *tnpS* and *tnpT* gene products. These products cannot complement resolvase mutations in other Class II transposons. Tn*4653* carries its own resolvase gene, *tnpR*, that when expressed results in the resolution of cointegrates formed by the Tn*1721* family of transposons. Indeed, the deduced amino acid sequences derived from the Tn*4653* and Tn*1721 tnpR* DNA sequences are identical. However, the same gene product cannot resolve Tn*4651* or Tn*4653* cointegrates. This paradox has been explained. In the first instance the Tn*4653* TnpR resolvase does not recognize the *res* site associated with the Tn*4651 tnpS* and *tnpT* genes. In the second instance the Tn*4653 res* region lacks the putative cross-over sequence, site I, for cointegrate resolution. Thus, the larger transposon Tn*4653* is dependent on the resolution functions of the smaller element, or on homologous recombination, to resolve cointegrate structures.

The sequences of the inverted repeats of Tn*4651* and Tn*4653* further support the classification of these elements. The Tn*4653* inverted repeats are 38 bp in length and are very similar to the Tn*1721* inverted repeats. The Tn*4651* inverted repeats show conservation of specific nucleotides associated with TnpA binding and nuclease activity (Ichikawa et al. 1990). However, they are 46 bp in length and have diverged significantly from the other Class II inverted repeats.

These studies have shown that the toluene catabolic transposons of pWW0 arose by transposition of a unique Class II element, Tn*4651*, into an element very similar to the Tn*1721* family of Class II transposons. The origin of the *xyl* operons (upper and lower) within the Tn*4651* element remains a mystery. These genes are readily deleted by homologous recombination between two direct repeat sequences of 1.4 kb, however no transposition functions have been associated with this module. The 1.4 kb repeated sequence may have been present in the primordial Class II element that gave rise to Tn*4651*, and also flanking the catabolic genes, therefore serving as a site for homologous recombination during the assembly of this element. Integration into Tn*4653* most likely occurred at a later time.

Plasmids specifying the degradation of toluene and xylenes have been isolated from locations around the world, from Japan to Wales (Shaw & Williams 1988). These plasmids vary in size, but the encoded pathways are very similar. The archetypal TOL plasmid pWWO undergoes a variety of genetic rearrangements, mediated by homologous recombination between repeated

sequences and by transpositional processes (Tsuda et al. 1989; Sarand et al. 1993). These rearrangements are not confined to the TOL-like plasmids alone; a segment of TOL-derived DNA has been found integrated into the chromosome of *Pseudomonas putida* (Sinclair et al. 1986). Selection for expression of the Tn*4651*-encoded *meta*-ring fission pathway can result in transposition of Tn*4651* into the plasmid pSAH of *Alcaligenes* sp. O-1 (Jahnke 1993). Hybridization experiments using different TOL plasmids suggest an evolutionary relationship among them, and the variety of the observed genetic rearrangements demonstrates the capacity for change conferred by transposition and recombination. Further discussion of the evolution of the pWW0 transposons Tn*4651* and Tn*4653* follows the description of the naphthalene catabolic transposon.

Tn*4655*, the naphthalene transposon

The NAH7 plasmid of *Pseudomonas putida* PpG7 was one of the first catabolic plasmids to be isolated, shortly after the camphor (CAM), octane (OCT) and salicylate (SAL) plasmids (Dunn & Gunsalus 1973). NAH7 belongs to the same *Pseudomonas* incompatibility group (IncP9) as the pWW0 (TOL) plasmid, and they share regions of similarity in the *sal* gene encoded *meta*-ring-fission pathway as well as in their plasmid replication and transfer regions (Barley et al. 1979; Lehrbach et al. 1983; Harayama et al. 1987). Recently it was reported that the naphthalene catabolic genes of NAH7 (*nah* and *sal*), like the toluene catabolic genes of pWW0, are part of a Class II transposon designated Tn*4655* (Fig. 1; Tsuda & Iino 1990). This 37.5 kb element is only capable of transposition in the presence of complementing Class II transposases from the Tn*1721* family of elements. The Tn*4653* transposase complements transposition of Tn*4655* to plasmid R388 at a frequency of about 10^{-3} per transconjugant, forming cointegrate structures with direct repeats of the entire 37.5 kb Tn*4655* element. Resolution of these cointegrates in *E. coli* HB101 required the product of the *tnpR* gene and *res*, located within Tn*4655*. The Tn*4655* *tnpR* gene product cannot complement resolution of other Class II transposon derivatives, nor can their resolvases function to resolve Tn*4655* cointegrates. The *tnpR* region of Tn*4655* occupies about 1.8 kb of the transposon, making it much larger than the typical Class II resolvase genes which are about 0.6 kb in length. Therefore the functional resolution system of Tn*4655* would appear to be unique. This is not true of the 38 bp inverted repeats of Tn*4655*, that differ at only 2 bp from the inverted repeats of Tn*4653* and that clearly belong to the Tn*1721* family of inverted repeats. Sequence similarity between the toluene and naphthalene catabolic transposons was found to extend beyond the inverted repeats, however hybridization studies using the Tn*4653* *tnpR* and *tnpA* genes as a probe failed to reveal similar sequences on Tn*4655*.

Speculations on the evolution of the toluene and naphthalene catabolic transposons

Many possible evolutionary schemes have been proposed for the assembly of the toluene catabolic plasmid pWW0 and its IncP9 relatives. These include speculations on the assembly of the upper and lower toluene catabolic operons and their regulatory determinants in a modular fashion (Cane & Williams 1986; Harayama et al. 1987; Keil et al. 1987; Shaw & Williams 1988; Horn et al. 1991). These ideas have gained further acceptance with the recent sequence information compiled for other aromatic hydrocarbon dioxygenases and ring-fission dioxygenases from various sources. The concept of the modular assembly of aromatic catabolic operons is discussed by van der Meer (1992) and elsewhere in this volume (Williams 1994).

Characterization of the naphthalene catabolic transposon Tn*4655*, and its comparison to Tn*4653*, prompted Tsuda & Iino (1990) to propose a scheme whereby Tn*4655* evolved independently of the toluene catabolic transposons from an ancestral Tn*1721*-like element. The minimum requirements for such a scheme were outlined as: 1) deletion or replacement of the original *tnpR* and *tnpA* genes of the Tn*1721*-like element, 2) incorporation of a novel resolution system, *tnpR'*, that now functions to resolve Tn*4655* cointegrates, and 3) incorporation of *nah* upper and lower pathway operons (Tsuda & Iino 1990).

Figure 2 presents one scheme for the evolution of these elements. The scheme is presented in order to illustrate possible steps in the evolution of these catabolic transposons, without intending to support one sequence of events over another. The premis for this scheme is that an ancestral IncP9 plasmid carried a Tn*1721*-like transposable element, and that the integration of two different Class II transposons into this element, along with the acquisition of catabolic gene modules, gave rise to the pWW0 and NAH7 plasmids as we see them today. This scheme is almost

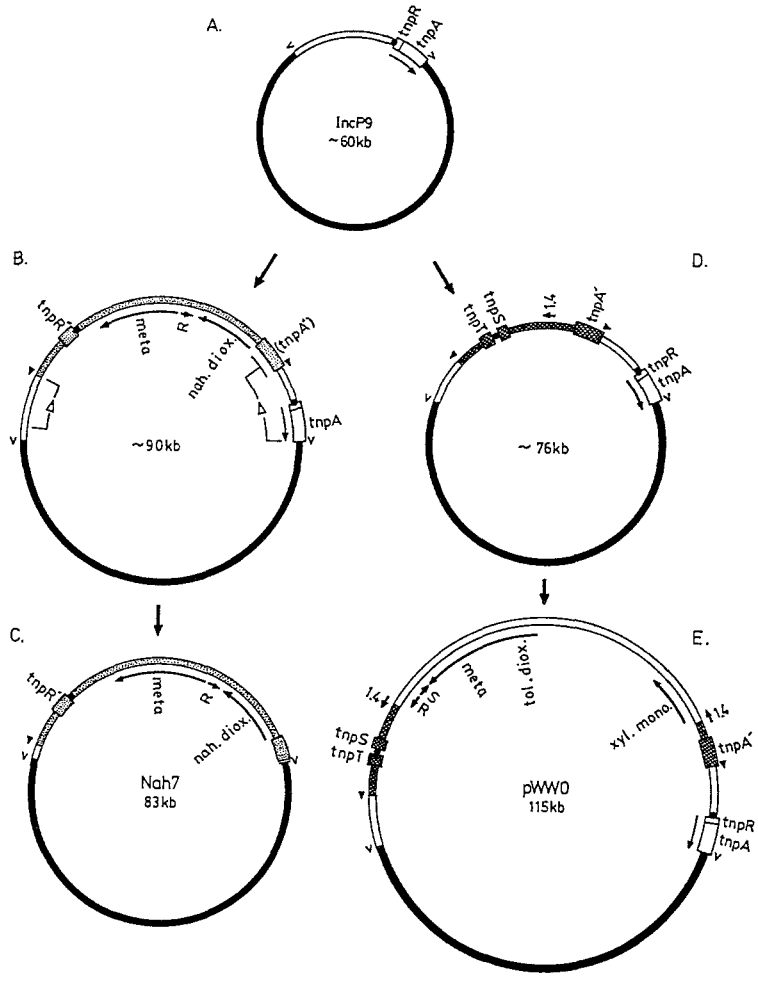

Fig. 2. Hypothetical scheme for the evolution of the Nah7 and pWW0 plasmids. A. An ancestral IncP plasmid carried a transposon related to the Tn*21*-family with resolvase (*tnpR*) and transposase (*tnpA*) genes and a *res* (•) locus. Inverted repeats for the Tn*21*-like ancestral element are indicated by <. Two separate integration events introduced transposons into the middle of the ancestral element to generate the intermediate structures shown (B. and D.). B. In the case of the Nah7 plasmid, the hypothetical integrated transposon carried the naphthalene catabolic genes in two operons: nah.doix., naphthalene dioxygenase operon (*nah*); meta, the *meta*-ring-fission operon (*sal*); both regulated by *nahR* (R). Two subsequent deletions (Δ) are required to eliminate most of the ancestral Tn*21*-like element, including its *tnpR* and *tnpA* genes, but not the inverted repeats. C. The Nah7 Tn*4655* transposon retains the divergent *tnpR'* of the hypothetical inner transposon. There is no sequencing information to show whether the inverted repeats (▼) and part or all of the *tnpA'* gene for the inner transposon remain. D. The pWW0 plasmid has retained all the important features of the nested transposons Tn*4651* and Tn*4653*. E. Acquisition of the toluene catabolic genes (xyl.mon., xylene monooxygenase; tol.diox., *meta*-toluate dioxygenase; meta, *meta*-ring-fission operon) by homologous recombination at the 1.4 kb sequence (1.4) is shown as the last step.

certainly true for the pWW0 plasmid, although at what step the catabolic gene operons were acquired is not known. The lower pathway *meta*-ring fission operons, and the *nah* and *xyl* genes for the upper pathways, may have been acquired by a series of recombination events either before or after the evolution of the transposition functions of the Tn*4653* and Tn*4655* elements (Tsuda & Iino 1990). The frequency with which long repeated sequences are found adjacent to the catabolic genes of large catabolic plasmids suggests that homologous recombination is an important mechanism for the assembly of catabolic operons. Direct and inverted repeat sequences are found adjacent to the catabolic genes of pJP4 (2,4-D), pAC25 and pSS60 (chlorobenzoates), pWW0 (toluene and xylene) and pTDN1 (aromatic amines) (Meulein et al. 1981; Ghosal et al. 1985; Burlage et al. 1990; Saint & Venables 1990).

The NAH7 scheme is more speculative and rests primarily on the observation that the nesting of Class II transposons occurs frequently, and that the Class II transposons represent target regions with enhanced frequencies of integration of various DNA elements (Grinsted et al. 1978, 1990; Priefer et al. 1981; Heffron 1983; Thomas & Smith 1987; Sherratt 1989; Thomas et al. 1992a). Evolutionary schemes that incorporate the formation of nested transposons and subsequent site specific or other deletions of internal DNA are well known for the antibiotic resistance elements (Grinsted et al. 1990). For example, Tn*21* contains an insertion of 11.2 kb of DNA specifying resistance to sulfonamide and streptomycin between the transposition loci (*tnpA, tnpR* and *res*) and the mercury resistance genes (*mer*). This internal element is flanked by 25 bp inverted repeats, outside of which are 5 bp direct repeats (Brown et al. 1986). Although this element has not been shown to be capable of independent transposition, it has the features of such an element. It is this internal element that contains the Integron region required for the site-specific integration of multiple antibiotic resistance genes into the Tn*21* family (Stokes & Hall 1989; also, see the discussion below of Tn*5271* and the structure of the IncPβ plasmids R751 and pBRC60).

Other evidence that supports the scheme presented in Fig. 2 is that at least 635 bp of DNA on the left (3′ to the upper and lower pathway orientations of transcription) and 50 bp on the right ends of Tn*4655* are nearly identical to the Tn*4653* sequence (Tsuda & Iino 1990). Characterization of the resolution locus of Tn*4655* also provides some support for this scheme. As discussed in the section on Tn*4655* the *tnpR* gene of this transposon is unlike any other resolvase characterized to date.

Both Class I and Class II elements promote intramolecular rearrangements. Intramolecular transposition inversions and deletions occur between a transposing Class II element and the target site. There is also evidence from *res* site sequences of both antibiotic resistance and catabolic Class II elements that site specific recombination between *res* sites of nested transposons can result in deletion of large parts of these elements (Grinsted et al. 1990). If there is selection at the organism level for maintaining a high frequency of transposition of catabolic genes then the inverted repeats of both elements of a nested pair should be conserved. However, the tranposition functions of either element may be lost. The *tnpA* and *tnpR* gene products may be provided in *cis* or *trans*, so that a nested transposon defective in one or both of these genes will continue to be transposable in the presence of a complementing element. Observations of Class II elements lacking resolution functions (IS*1071*); Nakatsu et al. 1991), transposition functions (Tn*4655*; Tsuda & Iino 1990) or both functions (IS*101*; Ishizaki & Ohtsubo 1985) support this conclusion. It can therefore be predicted that the inside element of a pair of nested transposons will tend to cause inversions or deletions of DNA within the borders of the outside element, rearranging this DNA or clearing it out entirely, while leaving the outside inverted repeats intact. Evidence to support this is found in the structures of Tn*951* (lactose) (Sherratt 1989; Grinsted et al. 1990), Tn*4655* (naphthalene) (Tsuda & Iino 1990), and in variants of Tn*4651*/Tn*4653* found on different TOL plasmids (Sarand et al. 1993).

The scheme presented in Fig. 2 for the evolution of the naphthalene catabolic transposon leads to the speculation that inverted repeats, and possibly a unique *tnpA* gene or a fragment of one will be found associated with an inside element that includes the unique *tnpR* gene. Additional sequencing within the Tn*4655* transposon, between the catabolic genes and the outside inverted repeats, is required in order to test this idea.

The variation evident in the well characterized toluene and naphthalene catabolic transposons likely represents only a fraction of the diversity that will be found in Class II elements specifying the degradation of environmental contaminants. As an indication of this it is clear that naphthalene catabolic genes are found on at least three different incompatibility groups of plasmids (IncP7, IncP9 and an unknown incompatibility group; Kochetkov & Boronin 1985).

Tn*5271*, the chlorobenzoate transposon

In 1988 we described *Alcaligenes* sp. strain BR60, isolated from runoff waters adjacent to a chlorobenzoate contaminated landfill. Deletion of chlorobenzoate catabolic genes from the indigenous plasmid pBRC60 (formerly pBR60) and recombination of plasmid sequences into the chromosome of this strain occurred at high frequencies (Wyndham et al. 1988). The chlorobenzoate catabolic genes were subsequently localized to a composite transposon designated Tn*5271* (Nakatsu et al. 1991). Tn*5271* is 17 kb in length and is flanked by 3.2 kb direct repeats designated IS*1071* (Fig. 1). While this element resembles Class I composite transposons in structure, characterization of the

flanking IS*1071* elements revealed that each is a Class II transposon. IS*1071* is 3,201 bp long and contains inverted repeat sequences of 110 bp. The outer 38 bp of the inverted repeats are related to the Class II inverted repeats that comprise the transposase recognition sequence. A single *tnpA* open reading frame occupies all but 35 bp at both ends of the DNA between the inverted repeats of IS*1071*. There is no *tnpR* gene or *res* site within IS*1071*, nor can similar sequences be found within Tn*5271*. The IS*1071* element mediates the formation of stable plasmid cointegrates in a *recA*$^-$ *E. coli* host. We have recently detected intramolecular transposition-inversion derivatives of the right IS*1071* element of Tn*5271* in *Alcaligenes* sp. BR60 and in an industrial isolate, CDC Group IVC-2 strain OCC13 (pOCC13) (Wyndham et al. 1994). The latter was recovered from an operating groundwater bioremediation system. These results demonstrate that IS*1071* is a functional transposon, and that the structural evolution of Tn*5271* is an ongoing process in a chlorobenzoate contaminated environment.

Conjugation of incompatible plasmids pGS65 or R68 into *Alcaligenes* sp. BR60 caused the recombination of Tn*5271* into the chromosome. The new chromosomal copy of Tn*5271* was detected by DNA hybridization. The complete transposon was shown to have integrated by mobilization experiments, in which pBRC40 (a deletion derivative of pBRC60) or R68 could mobilize the newly created chromosomal copy of Tn*5271* from BR60 into a chlorobenzoate catabolic mutants of *Alcaligenes* sp. BR60 (Ng & Wyndham 1993). In these studies, plasmid pBRC60 was shown to exhibit recombinational equilibrium in *Alcaligenes* sp. BR60, mediated by homologous recombination between plasmid and chromosomal copies of IS*1071*. A typical culture contained pBRC60 replicating as an episome in about 85% of cells, or integrated into the chromosome in about 15% of cells. Cells in which Tn*5271* was deleted from pBRC60 (yielding pBRC40), and integrated into the chromosome at an IS*1071* site, were also present at a high frequency.

The catabolic operon of Tn*5271*, designated *cbaABC*, encodes a 3-chlorobenzoate-3,4-(4,5)-dioxygenase and the associated reductase and dehydrogenase that convert 3-chloro- and 3,4-dichlorobenzoate to protocatechuate and chloroprotocatechuate (Nakatsu 1992; Nakatsu & Wyndham 1993). We have recently determined the sequence of the *cbaABC* operon in Tn*5271* and report similarities to the two-component oxygenases phthalate-4,5-dioxygenase, 4-sulfobenzoate-3,4-dioxygenase and vanillate demethylase (Nakatsu 1992).

A recent series of experiments demonstrated transposition of Tn*5271 cba* genes in *Comamonas acidovorans* (ATCC 15668) and *Comamonas testosteroni* (ATCC 11996), hosts that lack IS*1071* copies on the chromosome (Cashore 1993). The catabolic genes transposed to different sites in the chromosomes of these alternative hosts, however the amount of DNA integrated into the chromosomes of these strains extended from the *Eco*RI-4 to *Eco*RI-6 fragments of pBRC60, and including Tn*5271* (Fig. 3). In each case the amount of DNA transposed was the same. These results suggest that Tn*5271* is part of a larger, encompassing element that is mobile in natural pBRC60 hosts. We have tentatively assigned this element the designation Tn*5272*, awaiting characterization of the junction sequences between this element and *Comamonas* species chromosomal targets.

The organization of the determinants for replication (*oriV* and *trfA*), conjugal transfer (*oriT, pri* and *tra*), host-lethal (plasmid-inhibitory) functions and their suppression (*kil* and *kor*) and mercury resistance (*mer*) have been found to be conserved in the IncPβ (*Pseudomonas* IncP1) catabolic plasmids pJP4, pSS50 and pSS60, when compared to the archetype of this group, R751 (Burlage et al. 1990). These authors suggested that pBRC60 is also related to the IncPβ group, but that some variations in the backbone indicated a more distant relationship. Nakatsu (1992) has created a detailed map of pBRC60 and, using hybridization data presented by Burlage et al. (1990) and our own probing with IncPβ genes, we have shown that this catabolic plasmid has the same conserved arrangement of the major replication and conjugation genes as R751 (Fig. 3).

The locations of integration of the transposon Tn*402* (trimethoprim resistance) into R751 and the Hg^r determinants on pBRC60 are in similar locations. Also, the cryptic, Tn*21*-like transposon Tn*4321* is found in the same location on R751, between *oriV* and *trfA*, as Tn*5271* (Tn*5272*) on pBRC60 (Fig. 3; Sakanyan et al. 1985; Shoemaker et al. 1986; Smith & Thomas 1985, 1987; Cashore 1993). Another IncPβ plasmid, R906, contains a Tn*501*-like mercury, streptomycin and ampicillin resistance transposon in this same region of the plasmid. DNA sequences adjacent to the *oriV* region of the IncPβ plasmids R751 and R906, and the IncPα plasmids, have recently been compared (Smith et al. 1993). These alignments have identified a contiguous *trfA-oriV* segment represent-

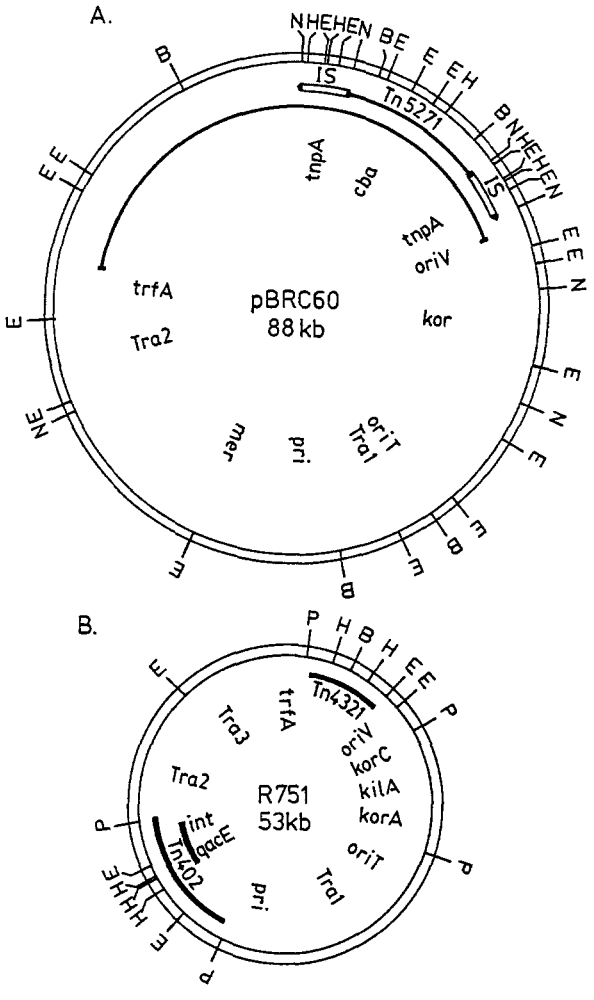

Fig. 3. Comparison of the plasmid maps of the IncPβ catabolic plasmid pBRC60 (A) and the resistance plasmid R751 (B). The pBRC60 map was constructed by C. Nakatsu in our laboratory (Nakatsu 1992). The R751 map was derived from the published map of Thomas & Smith (1987), with the addition of the Tn*402* integron from Paulsen et al. (1993). Loci common to both plasmids include: *kil*, host-lethal loci; *kor*, override of *kil* functions; *oriT*, origin of transfer; *oriV*, origin of vegetative replication; *pri*, primase; Tra, conjugal transfer functions; *trfA*, trans-acting replication factor. In addition, pBRC60 carries: *mer*, mercury resistance genes; *tnpA*, IS*1071* transposase genes; and *cba*, chlorobenzoate catabolic genes. The location of Tn*5271* is indicated, flanked by IS*1071* elements. The inner sector between *trfA* and *oriV* on the pBRC60 map indicates the approximate extent of DNA transposed into the chromosomes of *Comamonas acidovorans* and *Comamonas testosteroni* (Tn*5272*, see text). The R751 plasmid has an integron within Tn*402*, indicated by the line between *int* (integrase) and *qacE* (multi-drug exporter gene). The trimethoprim resistance locus *dhfrIIc* is located between these genes in the integron. The end points of Tn*402* are approximate. Restriction sites for E, *Eco*RI and H, *Hind*III are shown for both plasmids. Additional pBRC60 sites are: B, *Bgl*II and N, *Nhe*I. Additional R751 sites are: B, *Bam*HI and P, *Pst*I.

ing the ancestral IncP plasmid backbone from which the known IncP plasmids evolved. The region between *trfA* and *oriV* clearly represents a prefered target site for transposition of catabolic as well as resistance transposons. These observations support the conclusions reached by others concerning the existence of prefered target sites for transposition or recombination of DNA from other replicons into the IncPα and β plasmids (Cornelis et al. 1978; Priefer et al. 1981; Burlage et al. 1990); Thomas et al. 1992a).

Another feature of the IncPβ plasmid R751 that is important to this discussion is the discovery of an integron within Tn*402* (Paulsen et al. 1993; Fig. 3). Integrons were first recognized in the resistance plasmids R388 and R538-1, and the Tn*21* family of resistance transposons (Stokes & Hall 1989; Martinez & de la Cruz 1988, 1990; Grinsted et al. 1990). The explo-

sive evolution of the Tn*21* family of Class II resistance transposons is attributed to the formation of nested transposons and the action of the integrase system.

The integron that has been located within Tn*402* on R751 carries the trimethoprim resistance gene *dhfrIIc* and a multi-drug resistance locus designated *qacE* (Paulsen et al. 1993). The latter codes for a proton-motive-force dependent efflux protein active against antiseptics and disinfectants. QacE dependent efflux of ethidium, a polycyclic fluorochrome, is the assay used for this resistance mechanism. It is not difficult to envision that a similar activity might prove beneficial to microorganisms in the presence of toxic organic pollutant mixtures. To date, no similar integrase-dependent system for the acquisition of catabolic genes or toxic chemical efflux genes within the Class II transposons has been found. However, there appears to be no intrinsic reason why catabolic genes could not be assembled by a similar mechanism. The IncPβ catabolic plasmids related to R751 would be a logical place to look for such a system.

The evolution of composite IS*1071* transposons

The place of IS*1071* in the evolution of the Class II transposons was discussed previously (Nakatsu et al. 1991). IS*1071* was hypothesized to be similar to the common ancestral element giving rise to all Class II transposons. The properties of inter- and intramolecular replicative transposition of this element account for its distribution in multiple copies in the genomes of host bacteria (Ng & Wyndham 1993; Wyndham et al. 1994). These properties also suggest IS*1071* may be found to flank other composite transposable elements. Recently this was found to be true. A sequence of 710 bp upstream of the *dehH2* gene of plasmid pUO1 of *Moraxella* sp. strain B has been found to be identical to the IS*1071* sequence (Baum 1993). This region includes the N-terminal region of the *tnpA* gene and the 110 bp inverted repeat. Restriction mapping and unpublished sequence data discussed by Kawasaki et al. (1992) suggest that two copies of an IS*1071*-like element flank the *dehH2* dehalogenase gene on this plasmid (Fig. 4). These elements are directly repeated, as they are in Tn*5271*, resulting in high frequencies of deletion in both cases. It is of interest that the original host of this element, *Moraxella* sp. strain B, was isolated from industrial wastewater in Japan, while *Alcaligenes* sp. strain BR60 carrying Tn*5271* was isolated from an industrial landfill runoff in the Niagara River watershed.

An observation that has challenged the role of IS*1071* in transposition is the lack of 5 bp direct repeats of DNA flanking the left and right elements of Tn*5271* on plasmid pBRC60 (Nakatsu et al. 1991). These direct repeats are observed following transposition of all Class II elements and are a consequence of replication of the 5bp of DNA between the staggered nicks at the target site (Sherratt 1989). One hypothesis for the formation of IS*1071* composite transposons that explains the lack of 5 bp direct repeats is presented in Fig. 5. It shows a mechanism for the mobilization of chromosomal genes by an intramolecular transposition-deletion mechanism that would result in the 5 bp direct repeats being separated on the plasmid and chromosome. We suggest this mechanism is responsible for mobilizing the *cbaABC* operon into pBRC60, and possibly the *dehH2* gene into pUO1. This hypothesis is entirely consistent with the properties of IS*1071* (Nakatsu et al. 1991).

Tn*5280*, the chlorobenzene transposon

The catabolic plasmid pP51 was detected in a Rhine River sediment isolate, *Pseudomonas* sp. strain P51, capable of growth on chlorobenzenes (van der Meer et al. 1987). The catabolic genes on this plasmid are organized in two operons, *tcbAB* encoding chlorobenzene dioxygenase and chlorobenzene diol dehydrogenase, and *tcbCDEF* encoding chlorocatechol ring-fission enzymes (van der Meer et al. 1991a, 1991b, 1991c). The 5.1 kb *tcbAB* catabolic gene region is flanked by 1,142 bp insertion sequences, IS*1066* and IS*1067*, that differ at only one base pair within the 13 bp inverted repeat. The composite element has been designated Tn*5280* (Fig. 1; van der Meer et al. 1991c). IS*1066* shows similarity to the Rhsα elements of *Bradyrhizobium japonicum*, with 61.5% DNA sequence identity in the 1,010 bp region of the transposase open reading frame. There is also weak similarity to IS*630* of *Shigella sonnei*, with 21.8% identity over a 312 amino acid overlap in the transposase amino acid sequences. The IS*1066* and IS*1067* elements have sequences similar to the *Pseudomonas* -35 promoter region (TTGACA; Deretic et al. 1989) facing toward the inside of Tn*5280* and the *tcbAB* gene region. Whether this represents part of a functional promoter is unknown. The regulation of the catabolic genes of Tn*5280* is discussed further below.

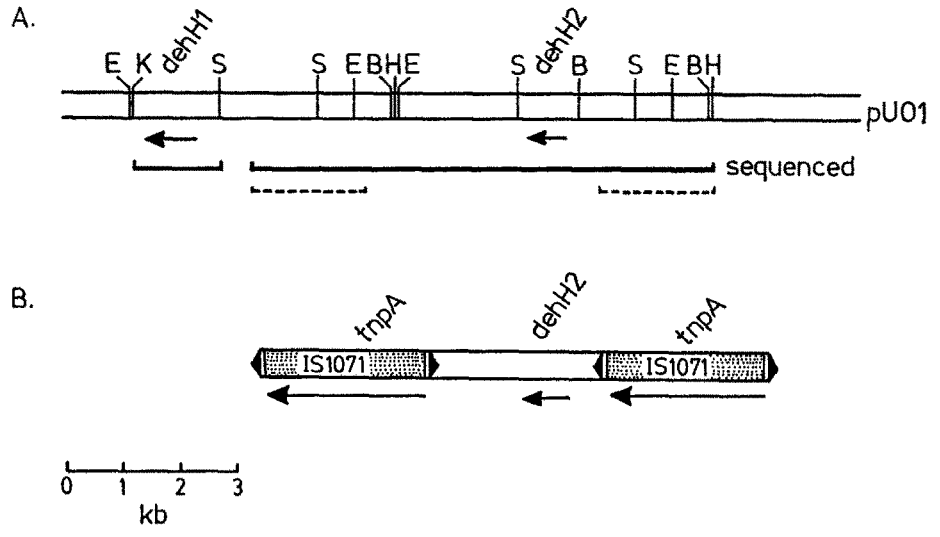

Fig. 4. Dehalogenase H1 and H2 genes and repeated sequences on plasmid pUO1. A. Restriction enzyme map of the region of pUO1 carrying the alkanoic acid dehalogenase genes *dehH1* and *dehH2* (Kawasaki et al. 1992). Restriction sites for E, *Eco*RI; K, *Kpn*I; S, *Sal*I; B, *Bam*HI; and H, *Hind*III are shown. The directions of transcription of the dehalogenase genes are indicted below the map. The region of the plasmid reported to be sequenced is indicated by the thick lines, and the region of repeated sequence by the dashed lines (Kawasaki et al. 1992). B. Proposed structure of the putative *dehH2* transposon, flanked by IS*1071*-like insertion sequences (coding the *tnpA* transposase), on pUO1. The proposed structure is based on the identity of sequences reported by Kawasaki et al. (1992) with the IS*1071* sequence reported by Nakatsu et al. (1991).

The composite Tn*5280* element, marked by insertion of a kanamycin resistance gene in *tcbB*, was shown to transpose in *Pseudomonas putida* KT2442 yielding single copy insertions at several points in the chromosome (van der Meer et al. 1991c). This transposition activity occurred at a frequency of between 10^{-6} and 10^{-7} per recipient.

The structure of the catabolic region of plasmid pP51, with Tn*5280* located approximately 3 kb from the lower pathway regulator *tcbR* and operon *tcbCDEF*, suggests that the IS elements were involved in bringing these two catabolic regions together on the plasmid (van der Meer et al. 1991c). This is a likely event, however we should look beyond the simple juxtaposition of the two elements for other phenotypic characteristics that might have provided a selective advantage to this arrangement. One suggestion has been that the combination of the two gene clusters on the same plasmid may have been necessary for their proper regulation (van der Meer et al. 1992). This idea is discussed further in the following section.

Regulation of transposable dioxygenase genes

The regulation of the upper and lower pathway toluene degradation genes on Tn*4651*/Tn*4653* by XylR and XylS, and of the naphthalene catabolic genes on Tn*4655* by NahR, has been recently reviewed (Nakazawa et al. 1990; van der Meer et al. 1992). The following discussion will focus on what is known about the regulation of the transposable chlorobenzoate and chlorobenzene catabolic genes.

The observation that *Pseudomonas* sp. P51 growing on chlorobenzenes is subject to occasional formation of toxic chlorinated intermediates that cause cell death emphasizes the importance of coordinated regulation of interconnecting pathways for aromatic compounds (van der Meer et al. 1991c). There is no direct evidence that a regulatory gene is included within the Tn*5280* transposon. The coding region of the *tcbAB* operon leaves virtually no room for a regulatory gene within the transposon. The *tcbR* gene product that regulates the expression of the *tcbCDEF* operon and that belongs to the LysR family of transcriptional activators (Leveau et al. 1994) does not interact with the promoter region of *tcbA* (van der Meer et al. 1991c). This shows that the two gene clusters must be independently regulated,

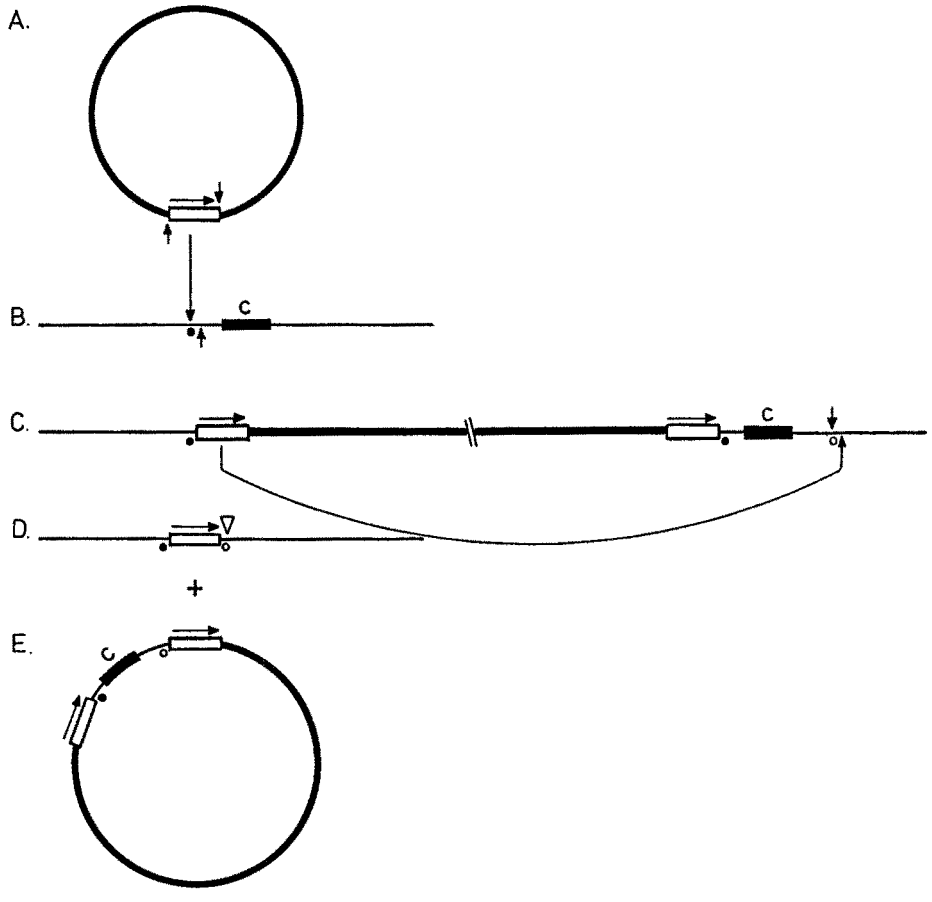

Fig. 5. Hypothesis for the formation of IS*1071*-flanked transposons involving sequential intermolecular and intramolecular transpositions. A. A plasmid like pBRC40 (Nakatsu et al. 1991) with a single copy of IS*1071* (open box) is nicked at the 3′-ends of the insertion sequence (short arrows) by the action of the *tnpA* gene product (direction of transcription indicated by the long arrow). B. The target site adjacent to chromosomal catabolic genes (c) is nicked, and the IS*1071* element is inserted and replicated by the usual Class II mechanism (Sherratt 1989), in the process replicating the 5 bp between the target site nicks (indicated by •). C. The integrated plasmid (thick line), flanked by direct repeats of IS*1071* (open boxes) and the 5 bp target site sequence (•). Intramolecular transposition (curved arrow) to a target site (indicated by o) on the other side of the catabolic genes (c) results in deletion of the plasmid and the catabolic genes. D. The chromosome is left with one copy of IS*1071* (open box) and a deletion of the catabolic genes (indicated by ∇). E. The deleted plasmid carries a composite transposon structure with direct repeats of IS*1071* (open boxes) flanking the catabolic genes (c). This structure is analogous to Tn*5271*. Note that the 5 bp duplications created by the two transposition events (• and o) are separated on the plasmid and chromosome in the final products.

however the nature of the TcbA dioxygenase and TcbB dehydrogenase regulation is unknown.

The catabolic transposon Tn*5271* carries a chlorobenzoate-3,4-dioxygenase operon consisting of three genes, *cbaABC*. These encode a two-component dioxygenase and a dehydrogenase (Nakatsu & Wyndham 1993; Nakatsu 1992). Sequence analyses outside of this operon, but within Tn*5271*, have failed to reveal an open reading frame with the characteristics of a positive regulatory element (Nakatsu, unpublished observations). When a *Not*I fragment from Tn*5271* containing the *cbaABC* genes alone was cloned into a *Comamonas acidovorans* ATCC 15668 host lacking the rest of Tn*5271*, the chlorobenzoate-3,4-dioxygenase genes were expressed in a regulated manner (Wyndham, unpublished observations).

Current data therefore suggest that control of the expression of the Tn*5271* and Tn*5280* dioxygenase operons involves the expression of regulatory genes located outside these elements. If so, then transposition and conjugal transfer to alternative hosts might be expected to lead to poor coordination of dioxygenase and lower pathway activities. This would be quite likely in the case of transposons transfered on broad-host-range plasmids into different genetic backgrounds. For plasmid pP51 the two gene clusters would

be transferred together, however pBRC60 carries the Tn*5271* dioxygenase genes separately from the lower, protocatechuate ring-fission pathway. The lower pathway is located on the chromosome of *Alcaligenes* sp. BR60 and the other hosts of this plasmid. These considerations lead to the hypothesis that the host ranges of these catabolic transposons may be limited by regulatory constraints. Evidence to support this idea was found in experiments investigating the transfer of pBRC60 from *Alcaligenes* sp. BR60 to the natural microbial community of continuous flow lakewater microcosms (Fulthorpe & Wyndham 1991, 1992).

There is a need for detailed studies of the factors that influence the distribution of mobile genetic elements in the environment. The most important factors influencing host range are likely to be the compatibility of the incoming replicon with the genome of the new host, and the regulation of the incoming metabolic pathway with respect to the expression of lower pathway or central metabolic pathway enzymes. It is apparent from a number of studies that regulatory constraints may be overcome in part by genetic rearrangements including the acquisition or deletion of DNA (Jeenes et al. 1982; Reineke et al. 1982). In some cases rearrangements effecting catabolic gene expression have been attributed to transposition (Scordilis et al. 1987; Kivisaar et al. 1989, 1990; Haugland et al. 1990; Jahnke et al. 1993; Sarand et al. 1993). The evidence that these processes have had a major influence on the evolution of catabolic pathways for xenobiotics has been reviewed recently (van der Meer et al. 1992).

Mobile genetic elements of unknown class

DEH, the dehalogenase element

The DEH element carries a gene for a single subunit dehalogenase active in the hydrolytic dehalogenation of 2-chloro- and 2,2-dichloro- acetic, propionic and butanoic acids (Slater et al. 1979; Thomas et al. 1992a, 1992b). This element was found in a soil *Pseudomonas putida* strain PP3 isolated following adaptation to growth in continuous culture on the herbicide Dalapon (2,2-dichloropropionic acid; Senior et al. 1976). The PP3 strain expresses two inducible dehalogenase proteins from genes designated *dehI* and *dehII*. Strain PP3 exhibited the unusual property of high frequency loss of either one or both dehalogenase activities under starvation stress or in the presence of toxic substrate analogues like trichloroacetic acid (Weightman et al. 1985). Under selection for growth on chlorinated alkanoic acids some of these dehalogenase-deficient mutants regained the inducible dehalogenase activities they had lost. A proposed hypothesis to explain these phenomena involved the activation of cryptic dehalogenase genes, possibly by transposition, and their deactivation by excision or loss of a regulatory element (Slater et al. 1985). An earlier report had implicated transposition in the transfer of the fraction I dehalogenase gene by R68.45 between *P. putida* strains (Beeching et al. 1983). Recently this dehalogenase gene, designated *dehI*, has been associated with a mobile genetic element designated *DEH* (Thomas et al. 1992a). Several IncP1 plasmids and the IncP9 plasmid pWW0 were transfered by conjugation into *Pseudomonas putida* PP3 and subsequently conjugated into *Pseudomonas putida* PaW340 with selection for growth on 2-chloropropionic acid. Transconjugants carried the *dehI* gene integrated into a number of different positions in the IncP1 plasmid RP4.5, and into at least two positions in the IncP9 plasmid pWW0. There was some indication that integration into RP4.5 was not entirely random but localized in the region of the defective Tn*1* element of this plasmid, however other integration points were observed. There was a clear indication of site specificity in the *Eco*RI-G fragment of pWW0. This fragment is located in a non-essential part of the large toluene catabolic transposon Tn*4653*, between the *tnpR* gene and the *tnpA* gene of the nested toluene transposon Tn*4651*. The other site of *DEH* integration into pWW0 was close to the *tnpS-res-tnpT* resolution determinants of Tn*4651*.

The amount of DNA from strain PP3 integrated into RP4-5 and pWW0 varied between 6 and 13 kb, and in at least one case where a 6 kb integration into pWW0 had occurred, tandem duplication of the *dehI* gene was observed. Amplification of the *dehI* gene may have been the cause of elevated dehalogenase I expression in *Pseudomonas putida* PP3 following prolonged selection in continuous culture (Weightman & Slater 1985).

When the DEH element was carried on either the unstable RP4 derivative plasmid pNJ5000 or the replication temperature sensitive derivative pMR5, the DEH element was observed to recombine into the chromosome of the host, *Pseudomonas putida* PaW340. From this location the element was capable of transposition to RP4 and conjugation into *Pseudomonas putida* KT2440 at a frequency comparable to the transposition frequency observed in the natural host, PP3 (approximately 10^{-4} per transconjugant). This fre-

quency was about three orders of magnitude higher than the frequency observed for Tn*5* transposition in the same plasmid donor/recipient combination. Transposition in *Pseudomonas recA* backgrounds was not demonstrated because of difficulties encountered with the stable maintenance of the donor plasmids in these strains. No segregation of the *dehI* and plasmid markers was observed in either $RecA^+$ or $RecA^-$ *E. coli* recipients, indicating that no movement of the DEH element from the unstable plasmid to the host chromosome occurred in this host.

The nature of the DEH element remains obscure. Information is required on the sequences of the ends of the different DNA fragments mobilized, and the sequences at the target sites of the element, before it can be determined whether this element moves by transposition, site-specific recombination or homologous recombination. The evidence to date supports a mechanism determined by the DEH element itself, but perhaps requiring host-encoded recombination functions. The preference for insertion of the DEH element into or near other transposons (Tn*4653* of pWW0 and the defective Tn*1* of RP4.5) is a characteristic of many transposable elements and insertion sequences. This property was discussed above in relation to the chlorobenzoate, toluene and naphthalene catabolic transposons.

*Tn*4371, *the chlorobiphenyl transposon*

Alcaligenes eutrophus strain A5 was isolated from PCB-contaminated reservoir sediments and shown to degrade 4-chlorobiphenyl (Shields et al. 1985). Originally this strain carried a 75 kb plasmid that contained 4-chlorobenzoate catabolic genes, thereby allowing complete mineralization of 4-chlorobiphenyl via the biphenyl *meta*-ring-fission pathway and the enzymes 4-chlorobenzoate dehalogenase, *p*-hydroxybenzoate hydroxylase and protocatechuate-4,5-dioxygenase. During transfer in the laboratory the region of the plasmid coding for 4-chlorobenzoate dehalogenase was lost and strain A5 now carries a 51 kb plasmid, pSS50, that carries neither chlorobiphenyl nor chlorobenzoate catabolic genes (Pettigrew et al. 1990; Layton et al. 1992; Springael et al. 1993a).

Recently the pSS50 plasmid was shown to mediate the transfer of 4-chlorobiphenyl catabolic genes to *Alcaligenes eutrophus* recipients at a frequency of about 10^{-5} per transconjugant (Springael et al. 1993a). These transconjugants harbour pSS50 derivatives carrying insertions of 59 kb of strain A5 chromosomal DNA. The transposing DNA, designated Tn*4371*, carries the determinants for 4-chlorobiphenyl metabolism to 4-chlorobenzoate, but not the chlorobenzoate dehalogenase gene. The *bphA, bphC* and *bphD* genes have now been localized by hybridization or subcloning to the middle of the Tn*4371* element (Springael 1994). Tn*4371* DNA was capable of transposition to plasmid RP4 with prefered target sites near the Tn*1* and IS*21* regions of this IncPα plasmid. Restriction mapping has shown the extent of Tn*4371* on RP4::Tn*4371* derivatives, however the junction sequences and transposition functions of this element have not been characterized.

A plasmid related to pSS50 from *Chromobacterium* sp. strain LBS1C1, isolated from the same PCB-contaminated reservoir as *Alcaligenes* sp. strain A5, was designated pSS60 (Layton et al. 1992). This plasmid carries the 4-chlorobenzoate dehalogenase gene in the region of the direct and inverted repeats of pSS50 (Burlage et al. 1990). Plasmid pSS60 has been shown to mediate the transfer of an element related to Tn*4371* to *Alcaligenes eutrophus* recipients. The resulting plasmids allow these recipients to convert 4-chlorobiphenyl to *p*-hydroxybenzoate, which is metabolized further by the chromosomal *p*-hybroxybenzoate hydroxylase and protocatechuate *meta*-ring fission pathway in these strains. Studies on the host range for expression of the RP4::Tn*4371 bph* genes have shown that growth on biphenyl can be confered on other *Alcaligenes* strains, an *Acinetobacter* species and fluorescent *Pseudomonas* species (Springael et al. 1993b; Springael 1994).

Indirect evidence from another source supports the idea that chlorobiphenyl catabolic genes have become widely dispersed because of their association with mobile genetic elements. The *bphC* genes sequenced from *Alcaligenes, Acinetobacter* and *Pseudomonas* species are very similar to one another and to a *bphC* gene recently isolated from a Gram positive *Arthrobacter* sp. M5 (Peloquin & Greer 1993).

Other rearrangements associated with catabolic genes

Many examples are known of instability in catabolic genes or of associations between catabolic genes and mobile DNA elements. However in most cases the catabolic genes have not been shown to transpose as defined elements. A 1,477 bp IS element designated IS*931* has been found flanking the 2,4,5-T catabolic genes of *Pseudomonas cepacia* strain AC1100 (Tomasek et al. 1989; Haugland et al. 1990).

The IS element has been shown to transpose on its own, suggesting a possible Class I composite transposon structure around these genes, however the whole element has not been shown to be mobile. Similarly, the nylon oligomer catabolic genes of plasmids pOAD2 and pNAD2 are associated with IS*6100* (Kato et al. 1994). IS*6100* is an 880 bp Class I insertion element originally found flanking the composite transposon Tn*610* (sufonamide resistance), of *Mycobacterium fortuitum* (Martin et al. 1990).

There is evidence from several sources that chlorocatechol *ortho*-ring-fission operons from different organisms are mobile. This was demonstrated nearly a decade ago for the *Pseudomonas putida* AC812 plasmid pAC27 (Chatterjee & Chakrabarty 1984) and has been supported by observations of tandem amplification of the homologous genes of *Pseudomonas putida* B13 (pWR1) (Rangnekar 1988). The chlorocatechol *ortho*-ring-fission genes of chlorobenzoate degrading *Pseudomonas putida* strain P111, which are located on the chromosome, have been observed to delete from the chromosome and to transpose to the indigenous 75 kb plasmid pPB111 of this strain (Brenner et al. 1993). The novel plasmid was designated pPH111. These rearrangements occurred following selection for growth on benzoate or 3,5-dichlorobenzoate, respectively. The nature of the element carrying the *clc* genes of strain P111 is unknown, however an estimate based on the amount of DNA acquired by plasmid pPB111 to generate pPH111 would indicate a size of 55 kb.

Experiments designed to select chlorobenzene utilizing recombinants from *Pseudomonas putida* R5-3 (pKFL2, toluene$^+$) and *Pseudomonas alcaligenes* C-0 (pKFL1, chlorobenzoate$^+$) parental strains have also provided evidence for the mobilization of catabolic genes (Kröckel & Focht 1987; Carney et al. 1989). The recombinant derivatives of strain R5-3, designated CB1-9 (pKFL3), had lost a 24 kb fragment of the original plasmid pKFL2 but had gained a chromosomal fragment from strain C-0 on the TOL-like plasmid. The nature of this element remains unknown.

Transfer of mobile genetic elements between replicons may also increase the flexibility of expression of catabolic genes. For example, enhanced expression of a poorly expressed β-lactamase gene, allowing growth of *Pseudomonas cepacia* on penicillin G, occurs as a result of transposition of insertion sequences from the chromosome to plasmid loci upstream of the catabolic gene (Scordilis et al. 1987). The ends of the toluene catabolic transposon (Tn*4652*, equivalent to Tn*4653* lacking the 39 kb toluene catabolic module) have been shown to alter the expression of plasmid genes encoding phenol monooxygenase and catechol-1,2-dioxygenase (Kivisaar et al. 1990).

Finally, there are several examples of catabolic genes that are readily excised from the genomes of their hosts. *Pseudomonas putida* strain RE204 contains a 105 kb plasmid, pRE4, that specifies the degradation of isopropylbenzene (Eaton & Timmis 1986a). A 20 kb region of this plasmid encoding isopropylbenzene-2,3-dioxygenase, isopropylbenzene dihydrodiol dehydrogenase and 3-isopropylcatechol-1,2-dioxygenase is readily lost by homologous recombination between repeated sequences flanking the catabolic genes (Eaton & Timmis 1986b). The aromatic amine catabolic genes of *Pseudomonas putida* mt-2 strain UCC22 (pTDN1) are also deleted at a high frequency (Saint & Venables 1990). The deleted DNA extends for 26 kb and is flanked by an approximately 1 kb directly repeated sequence that is a likely site for homologous recombination. The archetype of these excision events, the 39 kb toluene catabolic gene excision between 1.4 kb direct repeats on pWW0 (Meulein et al. 1981), is unrelated to the activity of the encompassing Tn*4651* and Tn*4653* elements. Therefore, in the absence of addition data showing transposition, or sequence information indicating the existence of transposition genes, one should view these excision events as simply an indication of instability and as an incentive to explore the surrounding DNA further.

Concluding remarks

The well characterized catabolic transposons are few in number, and yet they show a diversity of structure and nucleotide sequence that rivals that of the far greater number of antibiotic resistance transposons that are known. The research reviewed here has shown, above all, that catabolic transposons are an important source of genetic variation in bacteria degrading environmental pollutants. Part of this variation is generated by simple transposition, however equally important is the variation generated by the nesting of transposons, intramolecular transposition, and homologous recombination or site-specific recombination between DNA sequences within the Class I and Class II elements.

An important direction for future research on these elements is to assess their natural distribution and the regulation of their catabolic genes in different hosts. These studies will inevitably lead to the discovery of new catabolic elements and to a better understanding

of how these transposons have evolved under the selection pressure of environmental contamination. In turn these studies will contribute to improved strategies for the bioremediation of toxic organic contaminants by highlighting the critical importance of genetic adaptation in these processes.

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