TRANSPOSITION AND OTHER MUTATIONAL PROCESSES IN 

PSEUDOMONAS

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1. INTRODUCTION

The genus *Pseudomonas* represents the most diverse and ecologically widely distributed group of bacteria. Members of this genus are able to colonize many different environments, including soil and water, but also plant and animal tissues. Diversity arises and is maintained through interplay between ecological and genetic factors. Genetic variation is generated by point mutations and recombination events. Transposition is a recombination reaction that mediates the movement of discrete DNA segments between nonhomologous sites. Insertion of a transposable element into a gene usually inactivates it. However, many transposable elements have been shown to activate the expression of neighbouring genes. Multiple copies of the element can also act as substrates for homologous recombination that can lead to deletions, inversions, duplications or more complex alterations in the genome structure. Also, horizontally transferred genes can be frequently disseminated among bacterial populations as components of mobile genetic elements. The aim of this chapter is to give an overview of mobile DNA elements described in *Pseudomonas* species focusing on the mechanisms of transposon-associated genetic variation. The mechanisms of other mutational processes occurring in pseudomonads is also discussed.
2. TRANSPOSABLE ELEMENTS IN BACTERIA

Organisation of different transposable elements in bacteria is presented in Figure 1. The simplest bacterial transposable elements are insertion sequences (IS). Features and properties of IS elements are extensively

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**Figure 1.** Organisation of mobile elements in bacteria. A. IS elements and transposons. The terminal inverted repeats IRs that are binding sites for transposase are marked as grey boxes (IRL and IRR). IS elements are the simplest transposable elements. Two copies of the same IS element can form a composite transposon. Tn3 family transposons usually transpose by a replicative pathway forming an intermediate, called cointegrate. A site-specific recombination system
reviewed\textsuperscript{30}. IS elements are small and genetically compact. Generally they encode no other functions than those involved in their mobility. The majority of IS elements contain short inverted repeat sequences (IRs) ranging usually between 10 and 40 bp. IRs are specifically recognised and bound by an element-encoded transposase. IS elements like many other mobile DNA elements are flanked by directly repeated sequence (DR) of the target DNA. Target site duplications arise from staggered cleavage of the double-stranded DNA molecule. A given element will generally generate a DR of defined length. As a result of efforts in bacterial genome and plasmids sequencing, the list of IS elements is growing rapidly (see relevant database file at http://www-IS.biotoul.fr). According to their genetic organisation, similarities in their transposases, IRs, and generation of a DR of determined length, IS elements are classified into 19 families\textsuperscript{30}. Members of most individual families are distributed across many different eu-bacterial genera.

IS elements are able to form composite transposons. Well-known examples of composite transposons include a pair of IS elements flanking a segment of DNA, containing one or more genes encoding for functions like resistance to either antibiotics or heavy metals or catabolism of organic compounds. The transposition of a composite transposon is catalysed by the transposase encoded by one or both copies of the IS element. There are many IS elements (and composite transposons) that transpose as simple insertions.

\textbf{Figure 1. Continued}

(serine recombinase of the resolvase/DNA invertase family or tyrosine recombinase of the lambda integrase family) promotes the conversion of cointegrate into two separate components—the target with a simple insertion of the element and the regenerated donor replicon. The cointegrate resolution takes place at a \textit{res} site (shown as crosshatched box in the figure). Transposase and site-specific recombinase-encoding regions are indicated by black boxes. The striped boxes designate regions encoding other functions not related to transposition. B. Most conjugative transposons\textsuperscript{34} encode site-specific recombinases that belong to the lambda integrase family (black box in the figure). Conjugative transposons move via circular intermediate that is produced by excision of the integrated element from the donor chromosome. Some elements require for excision a specific protein analogous to lambda Xis protein. Excision and circularisation result in a closed junction \textit{(attP)} between left \textit{(attL)} and right \textit{(attR)} ends of the element. This junction \textit{(attP)} recombines with a target site \textit{(attB)} in the chromosome or on large plasmids. The circular form can transfer by conjugation to a new recipient cell in which reintegration can take place. C. Integrons\textsuperscript{183} are the recombinase-encoding genetic elements into which gene cassettes are integrated and excised. Gene cassettes contain a recombination site designated \textit{attC} and antibiotic resistance or other genes (striped boxes). Incoming cassettes are preferentially integrated at the \textit{attI} site. D. Group I intron mobility depends on an intron-encoded site-specific DNA endonuclease (grey box in the figure). The movement of a group II intron involves an RNA intermediate. In addition to the endonuclease activity, the group II intron-encoded proteins have maturase and reverse transcriptase activities\textsuperscript{10}. E. Retrons\textsuperscript{262} consist of three genes (indicated by grey box): \textit{msr} for the RNA-coding region, \textit{msd} for the msDNA-encoding region, and the reverse transcriptase-encoding region.
The Tn3 family transposons, in contrast to composite transposons, consist of a single transposable segment. They have similar IRs, generally of about 40 bp, and their transposition usually generates a 5-bp duplication of the target DNA. Like composite transposons, Tn3 family transposons include additional genes not related to transposition (e.g., resistance genes or catabolic operons). The Tn3 family transposons transpose using a replicative mechanism. However, some elements are capable of both replicative and conservative transposition. The organisation of transposons and transposition mechanisms of Tn3 family were recently reviewed.

Conjugative transposons are characterised by their ability to transfer laterally using a process that requires intercellular contact (see ref. [34] for a recent review). Conjugative transposons can excise from the chromosome, form a circular intermediate, and enter a new cell via conjugation. Unlike plasmids, the circular intermediate does not appear to replicate. Once in the recipient, the circular intermediate integrates into the recipient genome. Excision and integration mechanisms of conjugal transposons are similar to reactions catalysed by site-specific recombinases of temperate bacteriophages. Some conjugal transposons (e.g., Tn916) integrate into multiple sites in the genome of the recipient. Other elements utilise only a single target. Structural genes for tRNAs are common targets for insertions of bacteriophages and some conjugative transposons. Integrating genetic elements that use tRNA genes as targets create a duplication, restoring the tRNA gene.

Integrons are genetic elements that encode a site-specific recombination system which recognises and captures mobile gene cassettes (see ref. [183] for a recent review). A minimal integron consists of an integrase gene intI and an adjacent recombination site (attI). Integrons contain one or more non-autonomous mobile gene cassettes integrated at attI. The insertion of a gene cassette takes place by integrase-mediated site-specific recombination between the attC site of the circularised cassette and the attI site of the integron. Gene cassettes can be assembled into tandem arrays within the integrons. Many integrons, in turn, are located within transposons typically residing on broad host range conjugative plasmids, and are thereby frequently disseminated throughout a bacterial population by horizontal transfer.

Genomic islands are chromosomal gene clusters that appear to be derived from mobile sequences, although most of them are no longer mobile. They have no obvious characteristics other than those indicative of horizontally transferred loci. Genomic islands are found as compact, distinct units with a G+C content different from the remainder of the chromosomal DNA of the host. For recent reviews on genomic islands, see ref. [42] and [81]. The DNA flanking a genomic island often contains remnants of viral and transposase genes, like traces marking the way of acquisition of this genetic material. They are often large (30–100 kb), however, “microislands” made of one or a
few genes have been described as well. Genomic islands are sporadically present in some strains and absent in other strains within the same or related species. Depending on the functions that are encoded by genomic islands, they may be called pathogenicity islands (PAIs), symbiosis islands, or metabolic islands.

Prokaryotes contain reverse transcriptase-encoding retroelements that fall into two basic types: Group II introns that use reverse transcriptase (RT) to mobilise the intron element to new locations (discussed below), and retrons\textsuperscript{262}. Retrons encode RT and multicopy single-stranded DNA (msDNA). msDNA molecules consist of a short single-stranded DNA covalently linked to an internal G residue of the RNA molecule by a 2',5'-phosphodiester linkage. Initiation of cDNA synthesis begins at the 2'-OH group of the branching G residue, followed by cDNA synthesis using the same RNA transcript as a template. Although the function of msDNA is unknown, it has been hypothesised that retrons may be associated with bacterial pathogenicity because of their role in the enhancement of mutation rate in pathogenic bacteria during the host invasion\textsuperscript{125, 262}.

Group I and group II mobile introns are found in eubacteria and in eukaryotic organelles that have evolved from eubacteria (for a review, see refs [10] and [133]). It has been hypothesized that group II introns have migrated to the nucleus of eukaryotic cell, where they have evolved into spliceosomal introns as well as non-LTR retrotransposons and telomerase\textsuperscript{10}. Group I introns do not interrupt protein-coding genes in bacterial chromosomes (they interrupt only tRNA genes) in contrast to phage-encoded group I introns that always interrupt protein-coding genes, many of which are involved in some aspect of DNA metabolism\textsuperscript{133}. Most of the bacterial group II introns identified to date, interrupt known or putative IS elements and transposons and this is suggested to favour the spread of the introns within and between species\textsuperscript{54, 133}. Group I and II introns are capable of inserting into the same site in a cognate intronless allele (intron homing) or into novel genomic sites (intron transposition). Intron transposition, relative to intron homing, is an infrequent process due to the reduced affinity of intron-encoded proteins for variants of their recognition sequences and to heterology between intron-containing and intronless alleles\textsuperscript{54}.

3. TRANSPOSABLE ELEMENTS IN \textit{PSEUDOMONAS}

Most of the families of IS elements classified by Chandler and Mahillon\textsuperscript{30} are represented among natural isolates of \textit{Pseudomonas} strains (see http://www-is.biotoul.fr), whereas members of the IS5 family are most frequently recovered. There is also a number of IS elements characterised belonging to the IS3, IS21, and ISL3 families. Several members of IS elements described in \textit{Pseudomonas} are associated with composite transposons.
Notably, many catabolic transposons described in *Pseudomonas* are composite transposons\(^{231}\). However, the best-characterised catabolic operons in *Pseudomonas*, the toluene-degrading (*xyl*) genes on plasmid pWW0, are encompassed by Tn3 family transposons\(^{240}\). Also, many transposons carrying genes for mercury-resistance belong to Tn3 family\(^{74,101,145}\). Other mobile elements like conjugative transposons, integrons, class I and II introns, and gene islands are also represented in pseudomonads. Integrons, in turn, are frequently found within the Tn3 family transposons. Most of the composite catabolic transposons do not carry all the genes necessary for conversion of the initial substrates to the central metabolites. This contrasts with the Tn3 family catabolic transposons and the degradation islands.

3.1 **Transposable DNA Elements in the Genomes of**

*P. aeruginosa* PAO1 and *P. putida* KT2440

Search in the IS elements database at http://www-IS.biotoul.fr shows that the completely sequenced genome of *P. aeruginosa* PAO1 contains one copy of an IS110 family element and two distinct IS3 family elements, one of which is present in five copies and the other in a single copy. Also, 10 regions of PAO1 genome with sizes from 3 to 21 kb exhibited a significantly lower G+C content and unusual codon usage, possibly indicative of recent horizontal gene transfer\(^{221}\). There is also possibility that some genetic material has been acquired from an eukaryotic organism\(^{257}\).

Analysis of the complete genome sequence of *P. putida* KT2440\(^{153}\) has revealed the presence of seven novel multicopy IS elements, a Tn7-like element, and Tn4652 that was originally found in TOL plasmid pWW0\(^{242}\). These elements are listed in Table 1. Genome analysis of *P. putida* KT2440 has indicated that mobile elements have played a significant role in the acquisition of metabolic pathways. Around 80% of the chromosome shares a similar G+C content and oligonucleotide bias, whereas 105 islands showed atypical G+C content and/or oligonucleotide signature\(^{255}\). Twenty-nine of these islands carry a signature of mobile elements such as phages, transposons, IS elements, and group II introns, indicating a recent acquisition by horizontal gene transfer\(^{255}\).

3.2. **IS elements and Composite Transposons in Natural Isolates of** *Pseudomonas*

3.2.1. **Transposable Elements in Different Xenobiotic-Degrading Pseudomonads.**

Most IS elements associated with catabolic genes in *Pseudomonas* are the members of either the IS5 or the ISL3 family (Table 2). Additionally, there is
one prominent IS element, IS\textit{1071}, that formally belongs to the Tn\textsubscript{3} family but lacks a cointegrate resolution system\textsuperscript{30}. Notably, many IS elements listed in Table 2 and below have been identified only by their structural features. IS\textit{1071} was originally identified as an element flanking chlorobenzoate degradation genes (\textit{cba} genes) within the composite transposon Tn\textsubscript{5271} in a plasmid of \textit{Comamonas testosteroni} strain BR60150. This IS element has unusually long, 110-bp IRs. There is no significant homology of the IR sequence extending from 39 to 110-bp with sequences flanking the 38-bp IRs of the Tn3 transposons. Nakatsu \textit{et al.}\textsuperscript{150} have proposed that IS\textit{1071} may represent an ancestral element from which the Tn3 family has evolved by reducing the IRs and acquiring the site-specific recombination systems (resolvase or integrase), resistance determinants, or catabolic genes. IS\textit{1071} has been found to flank the different chlorobenzoate catabolic genes (\textit{clc}, \textit{cba}, or \textit{fsb}) in bacteria, and in many cases it forms a composite transposon\textsuperscript{47, 168}. Additionally, this element is linked to a diverse collection of other catabolic genes (Table 2). For example, ISP\textit{pu12}, which

\begin{table}[h]
\centering
\caption{Transposable elements in the genome of \textit{P. putida} KT2440.}
\begin{tabular}{lll}
\hline
Element & Family & Copy number \\
\hline
ISP\textit{pu8} & IS4 & 6 \\
ISP\textit{pu9}\textsuperscript{a} & IS\textsubscript{110} & 9 (2 truncated) \\
ISP\textit{pu10}\textsuperscript{a} & IS\textsubscript{110} & 7 \\
ISP\textit{pu11} & IS\textsubscript{110} & 2 \\
ISP\textit{pu13} & IS\textsubscript{66} & 2 \\
ISP\textit{pu14} & IS\textsubscript{66} & 6 \\
ISP\textit{pu15} & IS\textsubscript{66} & 5 \\
Tn\textsubscript{4652}\textsuperscript{b} & Tn3 & 1 \\
IS\textsubscript{1246}\textsuperscript{c} & IS5 & 1 \\
Tn7-like element & & 1 \\
Group II intron\textsuperscript{d} & & 8 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}The localisation of ISP\textit{pu9} and ISP\textit{pu10} in the chromosome of KT2440 indicates that these elements selectively target \textit{P. putida}-specific repetitive extragenic palindromic (REP) sequences\textsuperscript{153}.

\textsuperscript{b}Tn\textsubscript{4652} has transposed into the bacterial chromosome from TOL plasmid pWW0 during pWW0 elimination. This transposon is a derivative of Tn\textsubscript{4651} that has a deletion of catabolic region including \textit{xyl} genes as a result of reciprocal recombination between the two copies of IS\textsubscript{1246}\textsuperscript{184, 242}.

\textsuperscript{c}Locates within Tn\textsubscript{4652}.

\textsuperscript{d}Almost identical copies exhibiting a high homology to group II intron Xln6 found in \textit{P. alcaligenes} NCIB 9867\textsuperscript{269}. IS\textit{1071} was originally identified as an element flanking chlorobenzoate degradation genes (\textit{cba} genes) within the composite transposon Tn\textsubscript{5271} in a plasmid of \textit{Comamonas testosteroni} strain BR60150. This IS element has unusually long, 110-bp IRs. There is no significant homology of the IR sequence extending from 39 to 110-bp with sequences flanking the 38-bp IRs of the Tn3 transposons. Nakatsu \textit{et al.}\textsuperscript{150} have proposed that IS\textit{1071} may represent an ancestral element from which the Tn3 family has evolved by reducing the IRs and acquiring the site-specific recombination systems (resolvase or integrase), resistance determinants, or catabolic genes. IS\textit{1071} has been found to flank the different chlorobenzoate catabolic genes (\textit{clc}, \textit{cba}, or \textit{fsb}) in bacteria, and in many cases it forms a composite transposon\textsuperscript{47, 168}. Additionally, this element is linked to a diverse collection of other catabolic genes (Table 2). IS\textit{5}-family elements are widely distributed among \textit{Pseudomonas} strains and like IS\textit{1071}, they flank a variety of catabolic genes (Table 2). ISL3 family elements can form composite transposons, as shown in benzene-degrading \textit{P. putida} strain ML2 in which two copies of IS\textsubscript{1489} have formed composite transposon Tn\textsubscript{5542}\textsuperscript{58}. However, most of the ISL3 family elements discovered in \textit{Pseudomonas} have been identified due to insertions that have activated or inactivated certain catabolic genes (Table 2). For example, ISP\textit{pu12}, which
<table>
<thead>
<tr>
<th>IS element</th>
<th>Family</th>
<th>Host (location on plasmid)</th>
<th>Growth substrate (genes)</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS1071</td>
<td>Tn3</td>
<td>P. putida UCC22 (pTDN1)</td>
<td>Aromatic amines (tdnQTA₁A₂BR)</td>
<td>Flanks tdn genes at both sides</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas sp. ADP1 (pADP-1)</td>
<td>Atrazine (atzA, atzB, atzC)</td>
<td>Flanks each atz gene at both sides</td>
<td>[132]</td>
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<td></td>
<td></td>
<td>P. pseudoalcaligenes POB310</td>
<td>Carboxydiaryl ethers (pobAB)</td>
<td>Upstream of pobAB genes</td>
<td>[45]</td>
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<td></td>
<td></td>
<td>P. pavanaceae 170</td>
<td>1,3-dichloropropene (dhaA)</td>
<td>Multiple copies around dhaA</td>
<td>[171]</td>
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<td>IS2246</td>
<td>IS5</td>
<td>P. putida mt-2 (pWW0)</td>
<td>Toluene and xylenes (xyl operons)</td>
<td>Flanks xyl operons at both sides in Tn651</td>
<td>[184]</td>
</tr>
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<td></td>
<td></td>
<td>P. putida TMB</td>
<td>Xylenes and methyl-benzenes (tmb)</td>
<td>Locates in tmbU and tmbR spacer region</td>
<td>[56]</td>
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<td>IS1472</td>
<td>IS5</td>
<td>Pseudomonas sp. EST1001</td>
<td>Phenol (pheB₁)</td>
<td>Locates between pheBA genes and their promoter</td>
<td>[93]</td>
</tr>
<tr>
<td>IS5-like element</td>
<td></td>
<td>P. putida 01G3</td>
<td>Alkylbenzene (ebd operon)</td>
<td>Upstream of ebdA gene</td>
<td>[29]</td>
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<tr>
<td>IS5-like element</td>
<td></td>
<td>P. stutzeri AN10</td>
<td>Naphthalene (nah operons)</td>
<td>inpA1 downstream of nahD gene</td>
<td>[17]</td>
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<tr>
<td>IS5-like element</td>
<td></td>
<td>P. stutzeri AN10</td>
<td>Naphthalene (nah operons)</td>
<td>inpA2 downstream of nahW gene</td>
<td>[18]</td>
</tr>
<tr>
<td>IS5-like element</td>
<td></td>
<td>P. stutzeri AN10</td>
<td>Naphthalene (nah operons)</td>
<td>inpA3 upstream of nahW gene</td>
<td>[18]</td>
</tr>
<tr>
<td>IS5car1</td>
<td>IS5</td>
<td>Pseudomonas sp. CA10</td>
<td>Carbazole (car and ant genes)</td>
<td>Upstream of car operon, identical to IScar4</td>
<td>[156]</td>
</tr>
<tr>
<td>IS5car2</td>
<td>IS5</td>
<td>Pseudomonas sp. CA10</td>
<td>Carbazole (car and ant genes)</td>
<td>Upstream of antABC, differs from IS5car2 by 1 nt</td>
<td>[156]</td>
</tr>
<tr>
<td>IS5car3</td>
<td>IS5</td>
<td>Pseudomonas sp. CA10</td>
<td>Carbazole (car and ant genes)</td>
<td>Downstream of antABC</td>
<td>[156]</td>
</tr>
<tr>
<td>IS5car4</td>
<td>IS5</td>
<td>Pseudomonas sp. CA10</td>
<td>Carbazole (car and ant genes)</td>
<td>Downstream of car operon</td>
<td>[156]</td>
</tr>
<tr>
<td>IS1489v1</td>
<td>ISL3</td>
<td>P. putida ML2 (pHMT112)</td>
<td>Benzene (bed genes)</td>
<td>Upstream of bed genes, composite Tn (Tn5542)</td>
<td>[58]</td>
</tr>
<tr>
<td>IS1489v2</td>
<td>ISL3</td>
<td>P. putida ML2 (pHMT112)</td>
<td>Benzene (bed genes)</td>
<td>Downstream of bed genes, composite Tn (Tn5542)</td>
<td>[58]</td>
</tr>
<tr>
<td>IS element</td>
<td>ISL3</td>
<td>P. aeruginosa 142</td>
<td>Chlorobenzoates (ohb genes)</td>
<td>Downstream of ohb4AB genes</td>
<td>[239]</td>
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<tr>
<td>IS element</td>
<td>ISL3</td>
<td>P. aeruginosa JB2</td>
<td>Chlorobenzoate (ohb genes)</td>
<td>Upstream of ohb4AB genes</td>
<td>[239]</td>
</tr>
<tr>
<td>ISPv1</td>
<td>ISL3</td>
<td>P. stutzeri OX1</td>
<td>Xylenes (tou and xyl genes)</td>
<td>Switches tou and xyl genes on and off</td>
<td>[15]</td>
</tr>
<tr>
<td>ISPpu12</td>
<td>ISL3</td>
<td>P. putida mt-2 (pWW0)</td>
<td>Toluene and xylenes (xyl operons)</td>
<td>Inside Tn653, insertional inactivation of xyl genes</td>
<td>[260]</td>
</tr>
<tr>
<td>ISPpu12</td>
<td>ISL3</td>
<td>P. putida P3</td>
<td>Dichloropropionic acid (deh genes)</td>
<td>Composite Tn (DEH element), deh genes silencing</td>
<td>[254]</td>
</tr>
<tr>
<td>IS1411</td>
<td>ISL3</td>
<td>Pseudomonas sp. EST1001</td>
<td>Phenol (pheB₁)</td>
<td>Downstream of pheB₁ operon; activates silent genes</td>
<td>[93]</td>
</tr>
<tr>
<td>ISPpu1</td>
<td>IS630</td>
<td>P. putida (oleovorans) GPa1</td>
<td>Alkanes (alk genes)</td>
<td>Locates between alkL and alkB genes</td>
<td>[247]</td>
</tr>
<tr>
<td>ISPpu2</td>
<td>IS630</td>
<td>P. putida P1</td>
<td>Alkanes (alk genes)</td>
<td>Locates between alkS and alkB, flanks ISPpu2</td>
<td>[247]</td>
</tr>
<tr>
<td>ISPpu3</td>
<td>IS630</td>
<td>P. putida P1</td>
<td>Alkanes (alk genes)</td>
<td>Composite transposon TnPpu-alk1</td>
<td>[247]</td>
</tr>
<tr>
<td>ISPpu4</td>
<td>IS3</td>
<td>P. putida P1</td>
<td>Alkanes (alk genes)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Transposition and Other Mutational Processes in Pseudomonas**

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<table>
<thead>
<tr>
<th>IS element</th>
<th>Pseudomonadaceae sp.</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS110</td>
<td><em>P. putida</em> P1</td>
<td>Alkanes (alk genes) Downstream of alkT, ISPpu5 insertion in left end</td>
</tr>
<tr>
<td>IS1066</td>
<td><em>Pseudomonas sp.</em> P51</td>
<td>Chlorobenzene (tcbAB) Forms composite Tn with iso-IS element IS1067</td>
</tr>
<tr>
<td>IS21</td>
<td><em>Pseudomonas sp.</em> P51</td>
<td>Chlorocatechols (tcbR-CDEF) Remnants of IS element flank tcb cluster at both sides</td>
</tr>
</tbody>
</table>

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This IS element is associated with variety of aromatic or aliphatic components degradation genes in other bacteria (e.g., degradation of chlorobenzoates in *Comamonas testosteroni* BR60<sup>[50]</sup> and CPE3<sup>[47]</sup>; chloroacetate in *Delftia acidovorans* B<sup>[9]</sup>; o-phthalate in *Comamonas acidovorans* UCC61<sup>[52]</sup>; carboxydiaryl ethers in *P. pseudoalcaligenes* 2a<sup>[261]</sup> and in *Achromobacter xylooxidans* subsp. *denitrificans* strain EST4002<sup>(250)</sup>; p-sulfobenzoate in *Comamonas testosteroni* T-2<sup>[92]</sup>; aniline in *Comamonadaceae*<sup>[16]</sup>.

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Classified as IS5-like element by tnpA sequence analysis.

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Cloned as IS6-like element by tnpA sequence (designated as TnpA3) 95% identical to TnpA of IS1384<sup>[46]</sup>.

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tnpA sequence 98% identical to tnpA1 found downstream of nahD gene in *P. putida* AN1017<sup>[17]</sup>.

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IS1396-like element, 94% identical to IS1396-like element in *P. aeruginosa* 142<sup>[39]</sup>.

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IS1396-related element, TnpA 93% identical to TnpA of IS1396.

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IS1411 tnpA gene identical to the tnpA downstream of the chlorobiphenyl degradation genes bph in *Burkholderia cepacia* strain JHR22. Traces of IS1411 have also been found in the nitrotoluene-degrading *Pseudomonas* sp. strain TW3<sup>[90]</sup>.

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IS1600-related element; IS1600 forms composite transposon Tn3707 in *Ralstonia eutropha* chlorocatechol-degrading strain NH9<sup>[161]</sup>. 
originally lies on pWW0 in a region adjacent to the right end of Tn4651 (inside the right arm of Tn4653), can generate catabolic mutants by insertion into xyl genes on pWW0. Copies of ISPpu12 flank on either side the gene of hydrolytic dehalogenase (dehI gene) and its cognate regulatory gene dehR forming a composite transposon DEH able to transpose among a broad range of bacteria. The transposition frequency of the DEH element is unusually high, and transposition of either the composite transposon or ISPpu12 alone has shown to be associated with genomic rearrangements and gene silencing. Activation and inactivation of P. stutzeri OX1 methylbenzene catabolism pathways were mediated by ISL3-related element ISPs1 in response to growth substrate availability. Transposition of IS1411 became evident during the course of insertional activation of the promoterless pheBA genes in P. putida due to the presence of outward-directed promoters at the left end of IS1411. As a result of transposition, IS1411 leaves a copy in its original location. This DNA element can produce IS circles; therefore, it was supposed that IS1411 can follow a transposition pathway that utilises an IS circle as an intermediate.

A number of IS elements not directly associated with catabolic genes are also found in xenobiotic-degrading pseudomonads (Table 3). For example, P. alcaligenes NCIB 9867, isolated as dimethylnaphthalen degrader, carries in its genome.

### Table 3. IS elements from xenobiotic-degrading Pseudomonas strains not linked to catabolic genes.

<table>
<thead>
<tr>
<th>IS element</th>
<th>Family</th>
<th>Host (location on plasmid)</th>
<th>Growth</th>
<th>Comments substrate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS1383b</td>
<td>IS110</td>
<td>Pseudomonas sp. H (pPGH1; pPGH2)</td>
<td>Phenol</td>
<td>Two copies on pPGH1, four copies on pPGH2</td>
<td>[116]</td>
</tr>
<tr>
<td>IS1384c</td>
<td>IS5</td>
<td>Pseudomonas sp. H (pPGH1)</td>
<td>Phenol</td>
<td>Two copies on pPGH1 and on pPGH2; IRs are target for IS1383</td>
<td>[148]</td>
</tr>
<tr>
<td>IS1394</td>
<td>IS30</td>
<td>P. alcaligenes NCIB 9867 P25X</td>
<td>Xylenols</td>
<td>Ten copies in P25X chromosome</td>
<td>[264]</td>
</tr>
<tr>
<td>IS1474</td>
<td>IS21</td>
<td>P. alcaligenes NCIB 9867 P25X (pRA2)</td>
<td>Xylenols</td>
<td>13 copies in P25X chromosome, one on pRA2</td>
<td>[265]</td>
</tr>
<tr>
<td>IS1475</td>
<td>IS21</td>
<td>P. alcaligenes NCIB 9867 P25X</td>
<td>Xylenols</td>
<td>Targeted by IS1474</td>
<td>[265]</td>
</tr>
<tr>
<td>IS1491</td>
<td>IS21</td>
<td>P. alcaligenes NCIB 9867 P25X</td>
<td>Xylenols</td>
<td>At least 14 copies in P25X chromosome</td>
<td>[268]</td>
</tr>
</tbody>
</table>

a Carbon source used for strain isolation.
b Based on multiple alignments of the TnpAs, IRs and insertion sites of IS110 family elements, Lauf et al. defined a new family of IS elements, the IS1111 family which includes IS1383, IS1111, IS1328, and IS4321.
c TapA is 99% identical to TapA1 of P. stutzeri AN10 found downstream of nahD gene.
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genome multiple copies of various IS elements that may contribute to the tremendous plasticity of its genome\(^{264, 265, 268}\). Another example is phenol-degrading *P. putida* strain H, which carries on its plasmids six copies of IS element IS1383 and four copies of IS1384\(^{116, 148}\). Sequence analysis of IS1383 insertions has revealed that this element has high target specificity. IS1383 integrates into the IRs of IS1384 by a site-specific recombination event which utilizes a region that is formed after IS1383 circularisation\(^{148}\). Formation of the circular intermediate upon excision of IS1383 creates a strong promoter for the transcription of the *tnpA* gene whereas the spacer between the IRs of the element contains the \(-10\) hexamer of the promoter.

Several IS elements have been shown to form minicircle intermediates (e.g., IS911, IS21, IS3, IS1, IS2, and IS30)\(^{30}\). It is important to point out here that although some IS elements described above (e.g., IS1411 and IS1383) probably also transpose via the formation of IS circles as intermediates, this does not mean that circle formation could be applied to all IS elements.

### 3.2.2. IS Elements in Plant Pathogens

IS elements described in plant pathogens are listed in Table 4. IS801, a member of the IS91 family, was originally identified in *P. syringae*\(^{190}\). This element is widely distributed in

<table>
<thead>
<tr>
<th>Table 4. IS elements in plant pathogens.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Element</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>IS801(^a)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IS51</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IS1240</td>
</tr>
<tr>
<td>IS1240-like IS (IS3)</td>
</tr>
<tr>
<td>IS870-like IS (IS630)</td>
</tr>
</tbody>
</table>

\(^{a}\) Transposase genes nearly identical to IS801 *tnpA* have been identified in atrazine catabolic plasmid pADP-1 (four copies) from *Pseudomonas* sp. strain ADP1\(^{132}\) and in nitrobenzene-degrading *Pseudoalcaligenes* strain JS45\(^{53}\).
\textit{P. syringae}\textsuperscript{71}. Unlike most prokaryotic IS elements, IS\textsubscript{801} lacks terminal repeats, a trait shared by other members of the IS\textsubscript{91} family\textsuperscript{30}. IS\textsubscript{801} does not duplicate its target. The target sequences are highly specific, having partial homology to one terminus of the element\textsuperscript{187}. The IS\textsubscript{801} transposase is not a member of the DDE class transposases, and exhibits amino acid sequence homology to the transposases of \textit{E. coli} elements IS\textsubscript{91} and IS\textsubscript{1294} in conserved amino acid motifs identified in the replicases of certain plasmids that replicate as rolling circles\textsuperscript{187}. Like other IS\textsubscript{91} members, IS\textsubscript{801} possibly transposes via rolling-circle transposition (see ref. [67] for a review).

3.2.3. \textit{Transposition of IS}\textsubscript{21}. IS\textsubscript{21} is the prototype of a growing family of IS elements. IS\textsubscript{21} was originally identified in \textit{P. aeruginosa} antibiotic resistance plasmid R68\textsuperscript{258}. Mechanisms of transposition of this element have been extensively studied (for a review, see also refs [11, 76]). A single copy of IS\textsubscript{21} can generate simple insertions in a pathway that is thought to involve circularisation of IS\textsubscript{21}\textsuperscript{185, 200}. IS\textsubscript{21} spontaneously forms tandem repeats designated (IS\textsubscript{21})\textsubscript{2}. Plasmids carrying (IS\textsubscript{21})\textsubscript{2} can form cointegrates with other replicons at high frequency \textit{via} a cut-and-paste mechanism\textsuperscript{185}. The tandemly repeated copies of IS\textsubscript{21} promote insertion of the entire plasmid in a transposition event involving the abutted terminal IRs\textsuperscript{200}. IS\textsubscript{21} is the first transposable element for which a transposase/cointegrase specialisation has been reported\textsuperscript{200}. IS\textsubscript{21} contains two genes, \textit{istAB}, which are organised in an operon and encode transposition and helper proteins, respectively\textsuperscript{185}. When two IS\textsubscript{21} elements are organised in tandem, the abutted ends form a promoter that drives the expression of the \textit{istAB} genes in the downstream IS\textsubscript{21} element\textsuperscript{185}. IstB is needed for accurate strand transfer and capture of the target DNA\textsuperscript{199}. Full-length IstA is transposase, sufficient to carry out insertion of single IS\textsubscript{21} element as well as replicon fusion of (IS\textsubscript{21})\textsubscript{2} plasmids. In contrast, the truncated form of transposase, a cointegrase (expressed in frame from a natural internal translation start of \textit{istA}), essentially performs cointegrate formation but hardly any simple insertion\textsuperscript{200}.

3.3. \textit{Tn3 Family Transposons}

3.3.1. \textit{Tn3 Family Transposons in Catabolic Plasmids}. \textit{Tn3} family transposons present in catabolic plasmids of different \textit{Pseudomonas} strains are listed in Table 5. Transposition of \textit{Tn3} family catabolic transposons (e.g., \textit{Tn4653}, \textit{Tn4651}, and its derivative \textit{Tn4652}) have been extensively studied\textsuperscript{86, 87, 89, 245}; (for a review, see also ref. [240]). \textit{Tn4653} and \textit{Tn4651} (Figure 2A) are nested transposons: 70-kb \textit{Tn4653} spans 56-kb-long \textit{Tn4651}\textsuperscript{242, 243}. \textit{Tn4652}\textsuperscript{242} is a 17-kb derivative of \textit{Tn4651} that has a deletion of the catabolic region including \textit{xyl} operons as a result of reciprocal recombination between two copies of
Table 5. Tn3 family transposons in catabolic plasmids.

<table>
<thead>
<tr>
<th>Tn</th>
<th>Host (plasmid)</th>
<th>Catabolic substrate (genes)</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn4651&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>P. putida</em> mt-2 (pWW0)</td>
<td>Toluene, xylenes (xyl genes)</td>
<td><em>tnpS-res-tnpT</em> for resolution</td>
<td>[243]</td>
</tr>
<tr>
<td>Tn4653&lt;sup&gt;b&lt;/sup&gt;</td>
<td><em>P. putida</em> mt-2 (pWW0)</td>
<td>Toluene, xylenes (xyl genes)</td>
<td>Spans Tn4651, res defective, uses Tn4651 resolution system</td>
<td>[245]</td>
</tr>
<tr>
<td>Tn4655&lt;sup&gt;c&lt;/sup&gt;</td>
<td><em>P. putida</em> PpG7 (NAH7)</td>
<td>Naphthalene (nah genes)</td>
<td><em>tnpA</em> defective, site-specific integrase for cointegrate resolution</td>
<td>[244]</td>
</tr>
<tr>
<td>Tn4656&lt;sup&gt;d&lt;/sup&gt;</td>
<td><em>P. putida</em> MT53 (pWW53)</td>
<td>Toluene, xylenes (xyl genes)</td>
<td>Mutations in left IR decrease frequency of transposition</td>
<td>[241]</td>
</tr>
<tr>
<td>Tn4676</td>
<td><em>P. resinovorans</em> CAR1 (pCAR1)</td>
<td>Carbazole (car and ant genes)</td>
<td>Transposition system similar to Tn4651</td>
<td>[127]</td>
</tr>
<tr>
<td>ARMphe</td>
<td><em>P. fluorescens</em> (pAM10.6)</td>
<td>Phenol (<em>phe</em>BA)</td>
<td>Captured laterally transferred <em>phe</em>BA, lacks transposition functions</td>
<td>[169]</td>
</tr>
<tr>
<td>Tn5501e</td>
<td><em>Pseudomonas</em> sp. H (pPGH1)</td>
<td>None</td>
<td>Nearby phenol degradation genes (<em>phl</em>)</td>
<td>[115]</td>
</tr>
<tr>
<td>Tn5502</td>
<td><em>Pseudomonas</em> sp. H (pPGH1)</td>
<td>None</td>
<td>Nearby phenol degradation genes (<em>phl</em>)</td>
<td>[115]</td>
</tr>
</tbody>
</table>

<sup>a</sup>Transposition functions are not exchangeable with those encoded by Tn1722 subgroup transposons<sup>245</sup>. Deletion of catabolic region by reciprocal recombination between the two copies of IS1246<sup>184</sup> results in Tn4652<sup>242</sup>. Tn4652 transposase was not exchangeable with those of the Tn1722 subgroup transposons<sup>245</sup>.

<sup>b</sup>Transposition functions are exchangeable with those encoded by Tn1722 subgroup transposons<sup>245</sup>. Tn4654 is a derivative of Tn4653 that has deleted the catabolic region similarly to Tn4652<sup>243</sup>. Tn4654-related element has been involved in mobilisation of camphor degradation cluster in *P. putida* AC793<sup>126</sup> and similar element derived from alkanes degradation OCT plasmid of *P. putida* PpS5 was involved in mobilisation of non-conjugative replicons (discussed in Ref. [169]).

<sup>c</sup>Cointegration function is exchangeable with that encoded by Tn1722 subgroup transposons<sup>244</sup>.

<sup>d</sup>Transposition functions are exchangeable with those encoded by Tn1722 subgroup transposons<sup>241</sup>.

<sup>e</sup>Sequence nearly identical to Tn5501 was found in close proximity to the hydroxylaminobenzene mutase genes in *P. pseudoalcaligenes* strain JS45<sup>59</sup>. 
Figure 2. Genetic organisation of various transposable elements characterised in *Pseudomonas* strains. A. IS elements and transposons in TOL plasmid pWW0. Lines below the map indicate the extension of different transposons. The toluene degradation *xyl* genes (interrupted striped boxes) are located in Tn*4651*\(^{242}\) that is included within another transposon, Tn*4653*\(^{243}\). IRs of transposons are marked by grey boxes and functional res site for Tn*4651* and Tn*4653* by cross-hatched box. IRs for IS elements (IS1246, ISPpu12) are not shown. Genes encoding transposases (*tnpA*), site-specific recombinases (*tnpR, tnpS*) or proteins influencing activity of the formers (*tnpC, tnpT*) are shown by black boxes, and arrows indicate the direction of transcription of the genes. Note that sizes of the indicated genes and distances between these genes are not in scale. Tn*4632* and Tn*4634* can form as a result of reciprocal recombination between two copies of IS1246\(^{284}\) resulting in one copy of IS1246 element present in these transposons. B. Structural and functional organisation of Tn*5053*\(^{102}\) and Tn*402*\(^{176}\). The ends of transposons (t-end and i-end) are indicated by the grey box. Black boxes represent the genes encoding transposase (TniA), resolvase (TniR for Tn5053, TniC for Tn402) and auxiliary proteins (TniQ, TniB). DNA regions encoding other functions (mercury resistance operon in Tn5053 and integron In16 in Tn402 (striped boxes) are shown as well. Arrows indicate the direction of transcription of the genes. C. Schematic presentation of the *clc* genomic island.\(^{180}\) Integrase encoded by *intB13* near the right end of the *clc* element catalyses recombination between the 18-bp 3' end of the gly-tRNA (*attB* site) and the identical 18-bp sequence *attP* originating from the junction between left (*attL*) and right (*attR*) ends of the element. Excision of the *clc* element is also mediated by IntB13 integrase, although it is not yet clear whether other auxiliary factors may be needed for that process. Expression of the integrase gene in the circular form of the *clc* genomic island is controlled by a strong constitutive promoter present in the left end of the element.\(^{204}\) The striped box marks the chlorocatechol degradation gene cluster *clc* and arrow indicates the direction of transcription of the *clc* genes. The left end of the *clc* element contains an area well-conserved among various other genomic islands, and this region is supposed to be important for regulation of integrase expression (phage-related proteins, marked as striped box).
IS element IS1246\textsuperscript{184}. The deduced amino acid sequence of \textit{tnpA} from Tn4652 exhibited only a moderate homology to other Tn3 family transposases except the mercury resistance transposon Tn5041\textsuperscript{100}, suggesting that Tn4652 and Tn5041 are distant members of the Tn3 family forming a separate subgroup\textsuperscript{86}.

A site-specific recombination system for cointegrate resolution encoded by Tn4651/Tn4652 is also unusual among Tn3-like elements. Most members of the Tn3 family transposons use for cointegrate resolution a serine recombinase that is homologous to the prototypical resolvase TnpR of Tn3 catalysing only the intramolecular recombination between the two copies of the recombination site\textsuperscript{74}. A cointegrate resolution system of Tn4652 consists of two proteins, TnpS, which contains the R-H-R-Y tetrad commonly conserved in the integrase family of site-specific tyrosine recombinases, and TnpT, which does not show any significant sequence homology to the proteins that are involved in recombination\textsuperscript{69}. The 203-bp \textit{res} site partly overlaps the divergently transcribed \textit{tnpS} and \textit{tnpT} genes. Tn4652-encoded TnpS can catalyse both the intramolecular resolution and intermolecular integration reactions whereas TnpT most likely enhances the resolution frequency by interacting directly with TnpS\textsuperscript{69}.

Retrotransfer mediated by pWW0 has been demonstrated by Ramos-González \textit{et al.}\textsuperscript{178}. Retrotransfer has been defined as a conjugational biparental event that leads to the capture of genetic traits from the recipient by the original host of a conjugative plasmid\textsuperscript{228}. pWW0 mobilised some regions of the chromosomal DNA but not the others, suggesting that pWW0 may integrate into the genome of the recipient by a site-specific recombination\textsuperscript{178}. pWW0 with a mutation in the Tn4653 resolvase gene \textit{tnpR} was unable to mobilise chromosome, which indicates that the process of retrotransfer requires Tn4653 TnpR-provided site-specific recombinase activity\textsuperscript{192}.

### 3.3.2. Resistance Genes Associated with Tn3 Family Transposons and Integrons

Bacteria can become antibiotic-resistant either by spontaneous mutation or by acquisition of resistance determinants\textsuperscript{157}. Transposons and integrons encoding resistance to antibiotics are frequently carried on plasmids, but can also have a chromosomal location. Resistance transposons found in \textit{Pseudomonas} strains are listed in Table 6.

Antibiotic resistance and heavy metal resistance determinants are frequently contained in the same plasmid\textsuperscript{4}. Both plasmid-borne and chromosomal mercury resistance determinants often reside on transposons. A transposon originally found in enteric bacteria, the Tn3 family transposon Tn21, carries besides mercury resistance genes integron-associated genes for resistance to sulfonamide and streptomycin\textsuperscript{21, 75}. In \textit{Pseudomonas} strains, certain Tn21-related transposons also carry both mercury resistance and antibiotic resistance genes\textsuperscript{75}.
Table 6. Resistance genes found in transposons of different *Pseudomonas* isolates.

<table>
<thead>
<tr>
<th>Element</th>
<th>Host</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn5393&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>P. aeruginosa</em> MUS</td>
<td>Sm&lt;sup&gt;+&lt;/sup&gt; (strA-strB genes)</td>
<td>[223]</td>
</tr>
<tr>
<td>Tn1720</td>
<td><em>Pseudomonas</em> sp. strains&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sm&lt;sup&gt;+&lt;/sup&gt; (strA-strB genes)</td>
<td>[223]</td>
</tr>
<tr>
<td>Tn1404</td>
<td><em>Pseudomonas</em> sp. R171A</td>
<td>Tef&lt;sup&gt;+&lt;/sup&gt; (tetA gene)</td>
<td>[202]</td>
</tr>
<tr>
<td>Tn1403</td>
<td><em>P. aeruginosa</em></td>
<td>Integron In28 (blaP1, aadA2, cmlA1 cassettes, CbrSmrCmr) in res site, IS6100</td>
<td>[167, 252]</td>
</tr>
<tr>
<td>Tn405&lt;sup&gt;ab&lt;/sup&gt;</td>
<td><em>P. aeruginosa</em> Dalgleish</td>
<td>Carries Ch&lt;sup&gt;+&lt;/sup&gt;, Sm&lt;sup&gt;+&lt;/sup&gt;, Sp&lt;sup&gt;+&lt;/sup&gt;, Su&lt;sup&gt;+&lt;/sup&gt; determinants</td>
<td>[121]</td>
</tr>
<tr>
<td>Tn501&lt;sup&gt;c&lt;/sup&gt;</td>
<td><em>P. aeruginosa</em></td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;</td>
<td>[218]</td>
</tr>
<tr>
<td>Tn501v1</td>
<td><em>Pseudomonas</em> sp. BS6</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;; identical to Tn501</td>
<td>[145]</td>
</tr>
<tr>
<td>Tn501v2</td>
<td><em>Pseudomonas</em> sp. MU19-3</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;; identical to Tn501</td>
<td>[145]</td>
</tr>
<tr>
<td>Tn5050</td>
<td><em>Pseudomonas</em> sp. LS45-3</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;; belongs to Tn21 subgroup</td>
<td>[145]</td>
</tr>
<tr>
<td>Tn5051</td>
<td><em>P. putida</em> HU1-6</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;; belongs to Tn21 subgroup</td>
<td>[145]</td>
</tr>
<tr>
<td>Tn5061</td>
<td><em>P. alcaligenes</em> FA8-1</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;; belongs to Tn21 subgroup</td>
<td>[145]</td>
</tr>
<tr>
<td>Tn5041&lt;sup&gt;e&lt;/sup&gt;</td>
<td><em>Pseudomonas</em> sp. KHP41</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;; chimera of Tn21 subgroup and Tn4652-related elements, truncated group II intron</td>
<td>[100]</td>
</tr>
<tr>
<td>Tn5044</td>
<td><em>Pseudomonas</em> sp.</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;</td>
<td>[101]</td>
</tr>
<tr>
<td>Tn5053&lt;sup&gt;f&lt;/sup&gt;</td>
<td><em>Pseudomonas</em> sp.</td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;, tniABQ and tniR for transposition; targets res site</td>
<td>[103, 144]</td>
</tr>
<tr>
<td>Tn5563</td>
<td><em>P. alcaligenes</em> NCIB 9867</td>
<td>Encodes putative mercuric ions transport proteins</td>
<td>[266]</td>
</tr>
<tr>
<td>Tn402</td>
<td><em>P. aeruginosa</em></td>
<td>Integron In16, tniABQ and tniR for transposition; targets res site</td>
<td>[176, 144]</td>
</tr>
<tr>
<td>Tn2521</td>
<td><em>P. aeruginosa</em></td>
<td>Integron In33 (blaP1, aadA1 cassettes, Ch&lt;sup&gt;+&lt;/sup&gt;Sm&lt;sup&gt;+&lt;/sup&gt;/Sp&lt;sup&gt;-&lt;/sup&gt;) in res site, lacks transposition genes</td>
<td>[166]</td>
</tr>
<tr>
<td>Tn1696</td>
<td><em>P. aeruginosa</em></td>
<td>Hg&lt;sup&gt;+&lt;/sup&gt;, Su&lt;sup&gt;+&lt;/sup&gt;; integron In4 (aacC1, aadA2, cmlA1 cassettes, Gm&lt;sup&gt;+&lt;/sup&gt;/Sm&lt;sup&gt;+&lt;/sup&gt;/Cm&lt;sup&gt;-&lt;/sup&gt;) res site, IS6100</td>
<td>[167]</td>
</tr>
<tr>
<td>Tn1412</td>
<td><em>P. aeruginosa</em></td>
<td>Carries integron In3n res site of Tn5563-based backbone</td>
<td>[167]</td>
</tr>
</tbody>
</table>

<sup>a</sup>This element was originally isolated from *Erwinia amylovora*.<sup>3</sup>
<sup>b</sup>Strains A2, 7B12, and 8C32 carry Tn5393 in plasmids pPSR1, pPSR14, and pPSR15, respectively.
<sup>c</sup>Strains BixF6, PsR9, and PsR17 carry Tn5393 in plasmids pBIXF6, pPsR9, and pPSR17, respectively.
<sup>d</sup>Tn405 and Tn2521 are probably identical<sup>166</sup>.
<sup>e</sup>Tn5041-like elements have been found in many *Pseudomonas* strains<sup>101</sup>.
<sup>f</sup>Transposons identical or nearly identical to Tn5053 (variants v1, v3, v4 and v6 have been found in different *Pseudomonas* strains<sup>145</sup>).
Transposons Tn501, Tn5053, and Tn5041 are the best-studied mercury resistance transposons in *Pseudomonas* [22, 48, 100, 102, 144]. Mercury resistance transposon Tn5053 [102] transposes via replicative transposition. This transposon requires for cointegrate formation the activity of TniABQ, and TniR for the resolution of cointegrate [103]. Tn5053 is closely related to Tn402 (Tn5090) [176]. Transposons Tn5053 and Tn402 (Figure 2B) have been ascribed to a family distinct from the Tn3 family [103]. Based on the binding pattern and organisation of repeats at the ends of Tn402 [95] suggested that mechanism of transposition of Tn402 has similarity to Mu transposition. Most of the Tn5053/Tn402 family transposons have inserted into a common target site, a res region of Tn3 family elements upstream of the gene encoding a resolvase-like protein [144]. Not all Tn3 family transposons provide target sites for Tn5053. Testing of different targets for Tn5053 transposition revealed that res regions of Tn1721 and Tn5044 served as good targets, while another set of diverse res regions, such as those of Tn1 and Tn5053 itself, did not [144]. The res region served as a target for transposition of Tn5053 only in the presence of cognate resolvase expressed either in cis or in trans. Based on these findings the authors hypothesised that Tn5053 transposition may involve formation of an intermediate complex between the target DNA res region and the corresponding resolvase. This may also explain why the orientation of insertions of Tn5053 relative to the targeted res region was the same in all cases studied.

A detailed study of molecular diversity and evolutionary relationships of mercury-resistance transposons recovered from environmental bacteria has revealed that Tn21, Tn5053, and Tn5041-like transposons are widely distributed among *Pseudomonas* species all over the world [101, 145]. Some of the Tn5041-like transposons characterised were found to carry additional genetic material resulting from distinct recombination events, such as the insertion of group II intron INT5041C, a mer2 gene cassette or a Tn21 homologue [101]. Mosaic mercury-resistance transposons that have possibly evolved as a result of insertion of a Tn5053-like element into an ancestral Tn501-like transposon followed by deletions and other genetic rearrangements have been described in *P. stutzeri* plasmid pPB186. Other chimaeras of mercury resistance transposons formed as a result of homologous or site-specific recombination have been described [145, 270].

Antibiotic resistance genes are frequently found in gene cassettes that can be integrated at a specific position in an integron [182, 220]. Gene cassettes are the simplest mobile elements as they include only a single gene associated with a recombination site known as attC. Despite a substantial variation in length, these recombination sites have historically been referred to as 59-base elements (59-be). Among the three major classes of integrons described, the class 1 is most frequently encountered [183]. A number of Tn3 family transposons in *Pseudomonas* are known to carry a class 1 integron inserted into the res site of the transposon (Table 6). Class 1 integrons have been found also in soil bacteria, such as *P. stutzeri* [84] and *P. putida* [118].
3.4. Superintegrons

Ordinary integrons contain no more than three gene cassettes\textsuperscript{182}. Superintegrons may contain more than 100 gene cassettes encoding diverse functions including those possibly related to pathogenesis\textsuperscript{135}. Superintegrons were recently detected also in the genomes of \textit{Pseudomonas} species\textsuperscript{246}. Sequence analysis of the superintegron In55044 from non-pathogenic \textit{P. alcaligenes} strain ATCC 55044 revealed a high structural similarity to that described in \textit{Vibrio cholerae}\textsuperscript{135, 246}. A large fraction of ORFs was hypothesised to encode proteins associated with cell envelope, but no antibiotic resistance cassettes were found. Similar structures were detected in other \textit{Pseudomonas} species living in soil and aquatic habitats\textsuperscript{246}. Repetitive sequences that were identified as 59-be-like recombination sites in \textit{P. alcaligenes} superintegron In55044 form a family of elements that are distributed in \textit{Pseudomonas}. The authors proposed that a single susceptible ancestral element can be possibly present in all \textit{Pseudomonas} species, and has undergone sporadic expansion in some species while not in the others.

3.5. Conjugative Transposons and Genomic Islands in \textit{Pseudomonas} Isolates

Catabolic operons may be transferred to a new host also as part of conjugative transposons or genomic islands (Table 7). Among these genetic elements, a 105-kb-long \textit{clc} element (Figure 2C) of \textit{Pseudomonas} sp. strain B13, which carries genes for chlorocatechol degradation, is one of the best-studied examples in \textit{Pseudomonas}\textsuperscript{180, 248}. Transfer of the \textit{clc} element is mediated by a P4-related integrase. The \textit{clc} element integrates site-specifically into the chromosome of various bacterial recipients using the 3' end of glycine tRNA as an integration site. Transfer of the \textit{clc} element to other bacteria has been observed in complex microbial communities, such as sludges from soil and wastewater treatment plants\textsuperscript{234, 271}. Also, when the \textit{clc}-carrying \textit{P. putida} strain BN210 was inoculated into a bacterial population in 3-chlorobenzoate-contaminated wastewater, the \textit{clc} element was taken up by \textit{P. aeruginosa} strains and by strains belonging to the genera \textit{Ralstonia} and \textit{Comamonas}\textsuperscript{216}.

Genomic islands are unstable DNA segments that usually carry determinants important for the survival of the bacteria in unique environmental niches. Evolution of pathogenic variants from non-pathogenic or less virulent strains is a well-documented phenomenon in many bacterial species\textsuperscript{77}. Restriction mapping of genomes of \textit{P. aeruginosa} strains from diverse backgrounds has revealed that a significant amount of the variation among the isolates is due to insertions and deletions of large segments of genomic material\textsuperscript{191, 201}. Recent data have demonstrated that genomic islands play an important role in
### Table 7. Conjugative transposons and gene islands in different *Pseudomonas* isolates.

<table>
<thead>
<tr>
<th>Genetic element</th>
<th>Host</th>
<th>Function (genes)</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>clc element</td>
<td><em>Pseudomonas</em> sp. B13</td>
<td>Chlorocatechol degradation (<em>clc</em> genes)</td>
<td>105 kb, conjugative transfer, site-specific integration</td>
<td>[180]</td>
</tr>
<tr>
<td>bph-sal element</td>
<td><em>P. putida</em> KF715</td>
<td>Biphenyl and salicylate degradation (<em>bph, sal</em>)</td>
<td>90 kb, conjugative transfer, genetically unstable</td>
<td>[155]</td>
</tr>
<tr>
<td>Mobile element</td>
<td><em>P. aeruginosa</em> JB2</td>
<td>Hydroxy- and haloaromatics degradation (<em>hyb, clc</em>)</td>
<td>Conjugative transfer, <em>clc</em> genes flanked by IS21 family elements</td>
<td>[82]</td>
</tr>
<tr>
<td>Hrp PAI</td>
<td><em>P. syringae</em> strains</td>
<td>Type III secretion system, important in pathogenesis</td>
<td>50 kb, flanked by genetically diverse and unstable effector locus</td>
<td>[2, 36]</td>
</tr>
<tr>
<td>Glycosylation island</td>
<td><em>P. aeruginosa</em> PAK</td>
<td>Virulence factors glycosylation</td>
<td>16 kb, lcosylates only a-type flagellin present in pathogenic isolates</td>
<td>[6]</td>
</tr>
<tr>
<td>PAGI-1</td>
<td><em>P. aeruginosa</em> X24509</td>
<td>Most genes unknown, facilitates survival under stress</td>
<td>49 kb, has at least two different origins, present in many clinical isolates</td>
<td>[122]</td>
</tr>
<tr>
<td>PAGI-2&lt;sup&gt;b&lt;/sup&gt;</td>
<td><em>P. aeruginosa</em></td>
<td>Encodes metabolic functions and transporters</td>
<td>105 kb, hypervariable, integrated into tRNA&lt;sup&gt;Gly&lt;/sup&gt;</td>
<td>[114]</td>
</tr>
<tr>
<td>PAGI-3&lt;sup&gt;b&lt;/sup&gt;</td>
<td><em>P. aeruginosa</em></td>
<td>Encodes metabolic functions and transporters</td>
<td>103 kb, hypervariable, integrated into tRNA&lt;sup&gt;Gly&lt;/sup&gt;</td>
<td>[114]</td>
</tr>
<tr>
<td>Gene island</td>
<td><em>P. putida</em> IsoF</td>
<td>AHL signal molecules (<em>lasR-rsaL-lasI</em> homologues)</td>
<td>Integration mechanism unknown</td>
<td>[219]</td>
</tr>
</tbody>
</table>

*<sup>a</sup>clc Genes are almost identical to those described in chlorocatechol degradation plasmid pAC27<sup>35, 62</sup>.  
<sup>b</sup>Owns attachment sites and surrounding sequences similar to that found by analysing insertion sites of the *clc* element<sup>180</sup>. PAGI-2 is present also in *Ralstonia metallidurans* CH34 chromosome<sup>114</sup>.*
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Pathogenicity of *P. aeruginosa* (summarised in Table 7). Genetic material that contributes to parasitic fitness in plant pathogens\(^2, 36\) or enables production of \(N\)-acylhomoserine lactone (AHL) signal molecules\(^219\) can also be found in genomic islands (Table 7).

### 3.6. Mobile Introns in *Pseudomonas*

Group I and group II introns are found in bacteria and phages, but their distribution is erratic\(^54\). According to Edgell et al.\(^54\), group I introns are more frequent in bacteria than group II introns. Group II introns are not found in phages\(^54\). However, group II introns have been detected in a wider spectrum of phylogenetically diverse bacterial genera. Unlike eukaryotic group II introns, the bacterial group II introns described so far contain ORFs. A number of group II intron-encoded proteins contain reverse transcriptase and maturation domains but lack a recognisable endonuclease domain\(^133\). Most group I introns have been discovered in cyanobacteria; only a few cases have been reported in proteobacteria\(^54\). Recently, a novel group I intron that interrupts the anticodon loop of the tRNA\(^{Leu}\)(UAA) gene was described in genus *Pseudomonas* bacteria living in deep surface environments\(^251\).

Group II introns have been isolated in different *Pseudomonas* species. Introns discovered in *P. alcaligenes* NCIB 9867, *P. putida* NCIB 9869, and *P. putida* KT2440 are closely related\(^267, 269\). Although the *Pseudomonas* introns lacked an endonuclease domain, the group II intron found in *P. alcaligenes*, Xln6 and Xln3, were shown to have transposed from the chromosome to the plasmid\(^267, 269\). The fact that a group II intron can retrotranspose to new sites in a RecA-dependent fashion, in the absence of endonuclease activity, was experimentally demonstrated in the case of group II intron from *Lactobacillus lactis*\(^40\). A homologue of the *P. alcaligenes* group II intron Xln6 found in *P. putida* KT2440 (95% identity to the Xln6 sequence) was identified downstream of the *rpoS* gene in *P. putida*\(^269\). Eight almost identical copies of Xln6 homologue are present in the chromosome of KT2440 (Table 1). This indicates that after the transfer of Xln6 homologue to *P. putida*, this element was active and was possibly transposed into many sites.

### 3.7. Retrons in *Pseudomonas*

Retrons are found in a wide variety of Gram-negative bacteria and are responsible for the production of an unusual satellite DNA called msDNA\(^262\). A reference in the GenBank database\(^51\) indicates that an ORF found in *P. syringae* pv. *tomato* str. DC3000 may be a part of msDNA-producing retron.
4. INVOLVEMENT OF TRANSPOSABLE ELEMENTS IN MUTAGENESIS

Transposable elements contribute significantly to spontaneous mutagenesis in bacteria: insertional activation of adjacent genes, inactivation of genes by interruption, and rearrangement of the host genome are well documented\textsuperscript{30, 66, 128}. While transposition can cause a diverse set of mutations (most of them are potentially deleterious), a few favourable mutations may also occur, leading to adaptation of populations to a new environment. The following gives an overview of transposable elements-induced genetic changes that are involved in the genetic adaptation of \textit{Pseudomonas} (summarised in Table 8).

The positive effect of gene interruption by an IS element for the survival of bacteria under harsh environmental conditions was established in the case of transposition of an IS\textit{21}-family element IS\textit{S12} in \textit{P. putida} strain S12\textsuperscript{256}. This IS element was shown to play a key role in the tolerance of \textit{P. putida} S12 to sudden toluene stress by inactivating the transcriptional repressor of the solvent efflux pump genes srp\textit{ABC}.

\textit{P. putida} PP3 has evolved the ability to utilise halogenated alkanoic acids such as 2-mono- and 2,2-dichloropropionic acids (2MCP\textit{A}, and 2,2DCPA, respectively) during chemostat selection\textsuperscript{203}. This event led to the expression of two dehalogenases DehI and DehII, and associated permeases. The evolved \textit{P. putida} strain PP3 became sensitive to certain toxic, non-metabolisable substrates like dichloroacetic acid (DCA). Exposure of the strain PP3 to DCA led to the accumulation of DCA-resistant (and 2MCP\textit{A} negative) mutants caused by the movement of the gene for DehI within the transposon designated \textit{DEH}\textsuperscript{211, 235–237}. The ability to utilise 2MCP\textit{A} was readily restored under changed environmental conditions (in the presence of growth-supporting substrate 2MCP\textit{A})\textsuperscript{235}. As already discussed above, recent data have revealed that the \textit{DEH} element is a composite transposon on which \textit{dehI} and its cognate regulatory gene \textit{dehR}\textit{I} are flanked by copies of IS\textit{Ppu12}\textsuperscript{254}. IS\textit{Ppu12} is also present in TOL plasmid pWW0\textsuperscript{260} and its transposition is probably associated with many toluene catabolic mutants obtained during earlier studies on pWW0.

Activation and inactivation of \textit{P. stutzeri} OX1 methylbenzene catabolism pathways were also mediated by transposition of IS elements\textsuperscript{15}. \textit{P. stutzeri} strain OX1 was able to grow on toluene and \textit{o}-xylene as the sole carbon and energy source but \textit{m}-xylene and \textit{p}-xylene were not used for growth by this strain. Nevertheless, spontaneous mutants that had acquired the ability to grow on these substrates concomitantly lost the ability to utilise the ortho isomer appeared. Revertants that could utilise all the three isomers of xylene were isolated as well. The metabolic versatility in OX1 and its derivatives was brought about by genome rearrangements mediated by IS\textit{Ps1}: This DNA element can transpose into and precisely excise out of catabolic genes, causing inactivation or activation of respective catabolic functions\textsuperscript{15}. The authors supposed that
spontaneous switching one of the two alternative catabolic pathways (o-xylene catabolism vs m- or p-xylene catabolism) either on or off enables to avoid accumulation of toxic metabolites in mutant cells if a mixture of three xylene isomers is present in the medium.
Many IS elements and transposons carry outward-directed promoters or promoter modules (e.g., the \(-35\) hexamer)\(^{66,128}\). Transposition of such transposable elements upstream of silent genes can activate transcription of these genes. The Tn\textit{3} family transposon, Tn\textit{4652}, is a 17-kb derivative of the 56-kb toluene degradation transposon Tn\textit{4651}\(^{242}\). Tn\textit{4652} is found in the chromosome of \textit{P. putida} KT2440 and in its isogenic strain PaW85, and can activate transcription of silent genes by creating fusion promoters\(^{89,96,158}\). The fusion promoters are created as junctions between the \(-35\) hexamer provided by the terminal inverted repeats of Tn\textit{4652} and the \(-10\) hexamers in the target DNA for the transcription of promoterless phenol degradation genes in \textit{P. putida} strain PaW85. Analysis of the sequence of different fusion promoters has indicated that the DNA synthesis that occurred on transposition of Tn\textit{4652} might be mutagenic. Three insertions out of six different Tn\textit{4652} transposition sites studied contained point mutations in the copy of the direct repeat, which served as a sequence for the \(-10\) hexamer for the fusion promoter\(^{158}\). However, the point mutations identified were most likely under positive selection in starving population of bacteria selected for growth on phenol because they made the sequences of potential \(-10\) hexamers more similar to the consensus sequence of sigma\(^{70}\)-recognised promoters. Another mobile element, IS\textit{1411}, is also able to activate the promoterless phenol degradation genes \textit{pheBA} by inserting upstream of these genes\(^{93}\). Activation of transcription of the \textit{pheBA} genes occurred from outwardly-directed promoters present on the left end of IS\textit{1411}\(^{93}\).

Variations in the copy number of a mobile element can influence gene expression. For example, the \textit{clc} element is able to undergo amplification by producing multiple tandemly arranged copies\(^{179}\). Transfer of the \textit{clc} element into toluene-metabolizing \textit{P. putida} strain F1 created a hybrid pathway allowing the transconjugants to utilise chlorobenzene as a growth substrate. Amplification of the \textit{clc} element was a prerequisite for growth on chlorobenzene. The mechanism of tandem amplifications in F1 transconjugants is still unclear. The amplified structures in transconjugants were quite unstable and were deamplified under non-selective conditions by recombinational deletions between tandemly arranged copies. If deamplification had finally ended in two nonadjacent copies, no new amplification cycles occurred under selection for growth on chlorobenzene\(^{179}\).

Chromosomal rearrangements such as large inversions of DNA segments, caused in many cases by mobile DNA elements, may also be involved in adaptation of bacteria to a new environment. Such rearrangements have been frequently described among human pathogens\(^{88}\). For example, studies by Kresse \textit{et al.}\(^{110}\) have revealed that IS6100-induced large chromosomal inversions (LCIs) represent one of the mechanisms that can lead to the adaptation process of \textit{P. aeruginosa} to cystic fibrosis (CF) lung habitat. LCIs have played
a role in genome variability and adaptation to novel ecological niches also in other pseudomonads, for example, in *P. stutzeri*\(^7\).

Mobile introns present in bacterial genomes may serve as potential sources of change in genetic material as well. The involvement of group II introns in generation of catabolic mutants has been discussed by Yeo *et al.*\(^267\). *P. alcaligenes* catabolic mutants unable to degrade 2,5-xylenol contained genetic rearrangements possibly due to the presence of two copies of group II intron Xln6 surrounding 2,5-xylenol catabolic genes. Analysis of genetic rearrangements in the mutants indicated that these catabolic genes could be deleted as a result of recombination between the copies of Xln6. Disruption of genes for gentisate pathway by the intron Xln6 was also suggested in ref. [267].

5. REGULATION OF TRANSPOSITION IN PSEUDOMONAS

To avoid potentially deleterious effect of transposition to the host genome, the frequency of transposition in a cell is down-regulated, both by transposon-encoded and host-encoded factors. Transposition may be regulated by controlling transposase expression (transcriptional, translational, and/or post-translational control mechanisms) and also by factors that affect the transposition process itself (for a recent review, see ref. [30]). Under stressful environmental conditions, however, the increase in genetic diversity caused by a movement of transposable elements is one of the mechanisms allowing genetic adaptation of bacteria to a new environment\(^205\).

Increased transposition frequency may be the result of occasional inactivation of transposition control mechanisms but it can also be a regulated process. So far, there are only few published studies about regulation of transposition under stressful conditions. Study of the regulation of transposition of Tn\(3\) family transposon Tn4652 originated from TOL plasmid pWW0 is one of the best-studied examples in *Pseudomonas*. Transposition of Tn4652 seems to be restricted to *Pseudomonas* hosts because of a requirement of a *Pseudomonas*-specific host factor(s) for the transcriptional activation of the *tnpA* gene promoter\(^86\). The fact that some other groups have demonstrated transposition of Tn4652 in *E. coli*\(^104, 242, 245\) could be explained by the presence of putative promoter sequences upstream of the natural promoter of *tnpA* in constructs used in the transposition assay. The amount of Tn4652 TnpA is down-regulated by the Tn4652-encoded TnpC\(^87\). Transposition of Tn4652 is an exclusively stationary-phase-specific event and the maximum rate of transposition frequency of this element is achieved after few days of starvation of cells\(^89, 96\). Transposition of Tn4652 is strongly suppressed in *P. putida* defective for stationary phase-specific sigma factor RpoS: RpoS is required for
transcription initiation from the Tn4652 tnpA promoter of ref. [89]. Binding of IHF upstream to the tnpA promoter has also positive effect on the expression of the tnpA gene86. IHF binds both ends of Tn465286, 233. DNase I footprinting studies have revealed that binding of IHF to the ends of Tn4652 is necessary for transposase binding at the termini of Tn4652. Transposition of Tn4652 is drastically decreased in P. putida IHF-defective strain (H. Ilves, R. Hörak, R. Teras, and M. Kivisaar, unpublished results). Summing up, these results support the idea that at least in the case of some transposable elements, activation of transposition under stressful conditions can be an inducible process, not merely the malfunction of host defence mechanisms.

There are also other reports on studies of transposition in Pseudomonas demonstrating that transposition may be stress-induced. For example, it seems that some late-starvation signal can be needed for the activation of IS1411198. However, converse to Tn4652, the role of RpoS in regulation of transposition of IS1411 was opposite: Transposition frequency of IS1411 increased drastically in P. putida PaW85 RpoS-deficient mutant. Starvation-induced transposition has been also detected in the case of movement of DEH elements in P. putida strain PP3211, 235. Certain environmental conditions such as changes in temperature and pH affected the transposition frequency of these elements as well85. Unfortunately, studies of the molecular mechanisms of regulation of transposition of DEH elements are so far missing. The phenomenon observed by Slater et al.211 showing that trichloroacetic acid, which is neither the growth substrate nor the growth-inhibitor for cells of PP3, also drastically stimulates the transposition, would certainly deserve further investigations to elucidate the link between environmental signals sensed by bacterial cells and the frequency of transposition.

6. POINT MUTATIONS IN BACTERIA

Mutations not associated with the movement of transposable elements originate from various sources, including errors made during replication of undamaged template DNA, mutagenic nucleotide substrates, and endogenous DNA lesions129. Replication fidelity reflects the combined actions of DNA polymerase, proofreading exonuclease, and post-replication error-correcting DNA repair pathways. The integrity of DNA is continually threatened by ubiquitous agents such as heat, irradiation, reactive oxygen species and chemicals that break the DNA backbone or alter the chemical structure of DNA bases. Cells exposed to a variety of DNA-damaging agents result in a dramatic increase in the mutation rate64. DNA polymerase III may stall when attempting to copy a lesion. Continued unwinding of the DNA ahead of the blocked replication fork could provide a region of single-stranded DNA, allowing
assembly of an activated RecA nucleoprotein filament capable of inducing the SOS response. *E. coli* has five DNA polymerases. Three of these polymerases, pol II, pol IV, and pol V are induced as a part of SOS regulon in response to DNA damage\(^72\). These DNA polymerases can continue DNA replication when replication fork is collapsed at a blocking lesion, but the DNA synthesis by pol IV and pol V is error-prone\(^72,227\).

All organisms have multiple overlapping DNA repair pathways\(^55\). The three major DNA repair pathways are base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). Several additional pathways, including direct damage reversal, transcription-coupled repair, recombinational repair, and double-strand break repair also play important roles in preserving DNA integrity\(^195\).

### 6.1. RecA Functions in *Pseudomonas*

RecA protein is a multifunctional protein that is essential to three distinct but related biological processes such as general genetic recombination, regulation of the coordinated expression of many unlinked genes in response to DNA damage (SOS response), and the error-prone replicative bypass of DNA lesions (reviewed in refs [64], [72], [226], Figure 3). In *E. coli* SOS response, the expression of more than 30 proteins involved in DNA replication, repair, and control of cell division is induced after cells are exposed to DNA damaging agents\(^57,64\).

![Figure 3. RecA of *E. coli* is involved in homologous recombination, SOS induction, and error-prone replicative bypass of DNA lesions\(^64\). To function in these processes, RecA must assemble on ssDNA to form a nucleoprotein filament. The coprotease activity of RecA is necessary for catalysis of self-cleavage of LexA repressor and UmuD. UmuD forms a complex with UmuC. Only (UmuD)\(_2\)C complex resulting from cleavage of UmuD to UmuD functions as DNA polymerase (pol V). RecA nucleoprotein filament also targets the (UmuD)\(_2\)C complex at the site of a DNA lesion, thereby facilitating translesion DNA synthesis by (UmuD)\(_2\)C.](#)
The *P. aeruginosa* recA gene was shown to be capable of complementing recA mutations in *E. coli*\(^{109}\). Gene products with activities similar to that of the RecA protein of *E. coli* have been identified also in other *Pseudomonas* species, such as *P. putida*\(^{124}\), *P. fluorescens*\(^{44}\), *P. cepacia*\(^{151}\) and *P. syringae*\(^{83}\). Among these proteins, the RecA protein from *P. aeruginosa* has been most extensively studied. A distinct characteristic of the RecA protein from *P. aeruginosa* is its hyper-recombinogenic activity in *E. coli*. The *P. aeruginosa* RecA, when introduced into *E. coli* RecA-deficient mutant, increased the frequency of recombination exchanges about six times\(^{152}\). Subsequent studies\(^{8}\) suggested that the intensity of subunit–subunit interactions in the RecA filament structure can be one of the factors influencing the frequency of recombination exchanges by the RecA protein.

The RecA/ssDNA nucleoprotein filament functions as a coprotease that stimulates LexA autoproteolysis\(^{123}\). The resulting decrease in the cellular pool of LexA results in the induction of SOS regulon. LexA repressor binds the operators (called SOS boxes) of different SOS genes, which are also termed *din* (damage-inducible) genes\(^{99}\). The results presented in ref. [68] suggested that the *lexA* gene and protein are conserved in enterobacteria and pseudomonads. Conservation appeared also in the case of SOS boxes present upstream of the *lexA* genes compared in ref. [68]. The timing, duration, and level of expression can vary for each LexA-regulated gene, depending upon the location and binding affinity of the SOS boxes relative to the strength of the promoter. Therefore, some genes may be partially induced in response to even mild levels of DNA damage (e.g., of endogenous origin), while the other genes appear to be induced only if a high or persistent DNA damage is present in the cell (ref. [39] and references therein). Although there are some published reports on analysis of expression of DNA damage-induced promoters in *Pseudomonas*\(^{27,253}\), systematic studies have not been done.

### 6.2. Error-Prone DNA Polymerases in *Pseudomonas*

As mentioned above, the error-prone DNA polymerases pol IV (*dinB*(*P*) gene product) and pol V (encoded by *umuDC* genes) are induced as part of the SOS response. The *dinB*-encoded DNA polymerase IV (pol IV) belongs to the recently identified Y-family of DNA polymerases\(^{162}\). Like other members of this family, pol IV is involved in a translesion synthesis and mutagenesis\(^{63}\). DNA sequences homologous to *dinB* were found in the genome of a wide range of bacteria including representatives of *Pseudomonas* species\(^{162}\). Analysis of complete genome sequences of *P. putida* KT2440 and *P. aeruginosa* PAO1 indicates that putative pol IV homologues in these strains are closely related (77% identity), and have 50% identity with *E. coli* pol IV sequence.
Exposure of *E. coli* to UV or DNA-damaging chemicals causes a 100-fold increase in the mutation frequency. In the cells lacking functional pol V, UV-induced mutations occur only at a low frequency; these cells are also more sensitive to UV radiation than the wild type cells\(^{212, 226}\). The pol V-catalysed error-prone translesion synthesis takes place when all error-free processes to overcome replication block at a DNA lesion site have failed\(^{226, 232}\). It has also been argued that induced mutagenesis could help cells to survive periods of extreme environmental stress by acting as a mechanism of genetic adaptation of microbial populations\(^{174}\). Within Gram-negative bacteria, the genus *Pseudomonas* species examined so far lack chromosomally encoded pol V (for comparison of enterobacteria and pseudomonads, see also Table 9). Yet, many natural isolates of *Pseudomonas* strains contain plasmids that mediate UV-mutagenesis\(^{120, 138, 225}\).

Sundin *et al.*\(^{225}\) have isolated and characterised the plasmid-encoded *rulAB* genes in *P. syringae* that confer UV tolerance; these genes are supposed to play an important role in protecting bacteria in the phyllosphere from the DNA-damaging effects of UV-B radiation\(^{224}\). The *rulAB* genes were able to complement the *E. coli umuDC* mutant restoring the UV mutagenesis phenotype, which indicates that these genes may be functionally similar to the *E. coli umuDC*\(^{105}\).

Some antibiotic resistance plasmids, for example, R2, pPL1, R931, and pMG15 in *P. aeruginosa* strains were also shown to carry genes for UV mutagenesis\(^{119, 120, 208}\). Bacterial DNA is also a target for the stress of freezing and thawing, which introduces brakes in DNA strands\(^{5}\). Freezing and thawing has been shown to be mutagenic to a bacterium\(^{26}\). It is interesting to note that the studies on *P. aeruginosa* plasmid pPL1 have indicated that the genes that are responsible for protection against UV light can also protect the cells against X-ray and freeze-thaw damage\(^{259}\).

Genes conferring UV tolerance and UV-induced mutability have been localised on large catabolic plasmids in *P. putida*\(^{136}\). A 300-kb IncP2 plasmid CAM-OCT from *P. putida*, which carried genes for *n*-alkanes and camphor utilisation, enhanced both survival and mutagenesis after UV irradiation of *P. putida* and *P. aeruginosa* cells\(^{136}\). Interestingly, the UV response genes encoded by the CAM-OCT plasmid seem to be regulated differently from those present on other plasmids: The CAM-OCT plasmid enhanced both survival and mutagenesis in *P. aeruginosa* after UV irradiation by a RecA-independent mechanism\(^{137}\). Drawing parallels with other published data, there are several proteins induced by DNA damage in bacteria that are not directly regulated by RecA or LexA\(^{108}\). The mechanism of activation of respective genes on CAM-OCT plasmid, however, is still unknown. McBeth and Hauer\(^{138}\) have shown that the UV response genes cloned from CAM-OCT plasmid increased the frequencies of *P. putida* mutants capable of using new carbon sources such
### Table 9. Error-prone DNA synthesis and major DNA repair systems in enterobacteria and pseudomonads.

<table>
<thead>
<tr>
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<th>Enterobacteria&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pseudomonads&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td><strong>Error-prone DNA synthesis</strong></td>
<td>Genes for error-prone DNA polymerases</td>
<td>DNA pol IV is chromosomally encoded, <em>umuDC</em> homologues have been identified only in plasmids.&lt;sup&gt;105, 162, 225&lt;/sup&gt;. Mechanisms of regulation are unknown.</td>
</tr>
<tr>
<td>pol IV (dinB) and pol V (<em>umuDC</em>) located in the chromosome&lt;sup&gt;c&lt;/sup&gt; and are SOS-induced&lt;sup&gt;12&lt;/sup&gt;. The <em>umuDC</em> homologues have been identified in plasmids as well, and they may be differently regulated (see, e.g., ref. [111]).</td>
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<tr>
<td><strong>GO repair system</strong></td>
<td>MutT, MutM, and MutY work in concert to overcome the effects of oxidative damage of guanine (GO)&lt;sup&gt;141&lt;/sup&gt;.</td>
<td>Functional homologues to <em>E. coli</em> MutT, MutM, and MutY have been reported&lt;sup&gt;165, 198&lt;/sup&gt;.</td>
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<tr>
<td><strong>MMR</strong></td>
<td>MutH endonucleolytic activity is activated by a complex of MutS, MutL, and mismatched DNA&lt;sup&gt;30, 131&lt;/sup&gt;. UvrD helicase unwinds the duplex DNA molecule at the nick.</td>
<td>MutH-encoding homologue is lacking. <em>P. aeruginosa</em> <em>mutS</em>, <em>mutL</em>, and <em>uvrD</em> are unable to complement <em>E. coli</em> MMR-deficient mutants&lt;sup&gt;163&lt;/sup&gt;.</td>
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<tr>
<td><strong>NER</strong></td>
<td>UvrA, B complex scans DNA damage, UvrB and UvrC are endonucleases that cut DNA on different sides of the lesion&lt;sup&gt;196&lt;/sup&gt;. UvrD helicase unwinds the damaged oligonucleotide.</td>
<td>Homologues to <em>E. coli</em> genes encoding NER have been reported, but some of the <em>uvr</em> genes may be differently regulated&lt;sup&gt;188&lt;/sup&gt;. Activities of UvrD in NER and MMR might be different&lt;sup&gt;163&lt;/sup&gt;.</td>
</tr>
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</table>

<sup>a</sup>Results obtained from studies of *E. coli* and *Salmonella typhimurium* are summarised.

<sup>b</sup>Results obtained from studies of *P. aeruginosa*, *P. putida*, and *P. syringae* are summarised.

<sup>c</sup>*E. coli* that has been used for the study of role of pol IV in mutability, carries two copies of *dinB*, one in the chromosome and one in the F<sup>+</sup> plasmid<sup>139</sup>.
as ethyl lactate or 3-methyl-3-buten-1-ol approximately by one order of magnitude. Hence, the presence of genes responsible for UV mutagenesis on a catabolic plasmid might give a bacterial cell a potential to expand its growth substrate range also in natural environments. A large catabolic plasmid TOL plasmid pWW0 in *P. putida* also confers UV-induced mutability. The genes conferring UV mutability are able to complement *E. coli* pol V-deficient mutant (M. Tark, K. Tarassova, G. Kivi, R. Tegova, A. Tover, and M. Kivisaar, unpublished results).

### 6.3. DNA Repair Pathways in *Pseudomonas*

Reactive oxygen intermediates are produced during aerobic respiration and other metabolic processes. Also, pathogenic bacteria are exposed to exogenous reactive oxygen intermediates during infection of the host\(^{143}\). Defence against oxidative stress is provided by multiple mechanisms including detoxification of reactive oxygen species and repair of oxidatively damaged DNA. *P. aeruginosa* is the first micro-organism for which a genetic link between an oxidative stress gene and a DNA repair gene has been observed\(^{160}\). A *P. aeruginosa* *oxyR* homologue, which encodes one of the key regulators modulating oxidative stress in bacteria, was found in an operon with *recG*, encoding a homologue of the *E. coli* RecG helicase, which is an ATP-dependent DNA recombinase implicated in DNA replication, recombination, and repair\(^94\). The *P. aeruginosa* RecG-deficient mutant was hypersensitive to oxidative stress and UV irradiation\(^{160}\).

Bridges et al.\(^{20}\) have proposed that oxidized guanine residues, including 7,8-dihydro-8-oxoguanine (GO), constitute an important source of spontaneous mutation. To protect their genomes from oxidative DNA damage, bacterial cells have evolved efficient repair systems including DNA glycosylases MutY, MutM (Fpg), and MutT protein which hydrolyses GO\(^{141}\) (Figure 4). The function of MutM glycosylase is to remove mutagenic GO from the damaged DNA, and MutY glycosylase provides defence by removing A misincorporated opposite GO or G following DNA replication\(^{141}\). Cells that lack activity of these glycosylases have elevated rates of G:C to T:A transversions\(^{154}\). The GO repair system has mainly been studied in *E. coli*, but the results of some recently published papers indicate that the GO repair system is an important defence system against the oxidatively damaged DNA also in *Pseudomonas* species\(^ {165, 198}\). The *mutT*, *mutM*, and *mutY* genes of the GO repair system of *P. aeruginosa* PAO1 were able to complement the corresponding *E. coli* GO repair-deficient strains\(^ {165}\) (Table 9). The deduced amino acid sequence of *P. putida* *mutY* homologue exhibited 78% identity with MutY sequence of *P. aeruginosa*, and the frequency of spontaneous Rif\(^{\text{r}}\) mutants increased approximately 60-fold in the MutY-defective strain PaW85 compared to the
Figure 4. GO repair system in bacteria. Several enzyme activities work in concert to overcome the effects of oxidative damage of guanine (GO). A. Oxidised guanine 8-oxodGTP is removed from the deoxynucleotide pool by MutT, which hydrolyses 8-oxodGTP to 8-oxodGMP. B. When a GO lesion has occurred in DNA, DNA synthesis past GO can result in the misincorporation of adenine opposite the damaged guanine. MutM glycosylase removes mutagenic 8-oxoG from the damaged DNA. The glycosylase has an associated AP (apurinic) endonuclease activity that cuts the phosphodiester backbone. Exonuclease processing of the gap generated by MutM creates a substrate that can be repaired by DNA polymerase I and ligase. If MutM does not remove 8-oxoG from DNA, DNA synthesis past GO (replication 1) may result in the misincorporation of adenine opposite GO. An adenine DNA glycosylase MutY (also with associated AP endonuclease activity) removes the misincorporated adenine from A:GO mispair. Repair synthesis then results in the formation of a structure with C paired with GO, which is the substrate for MutM. If MutY does not remove A that has mistakenly paired with 8-oxoG, the second round of DNA replication (replication 2) will fix the G:C to T:A transversion (DNA polymerase inserts correct T opposite A). C. If replication occurs with 8-oxodGTP in the deoxynucleotide pool, inaccurate replication can result in either the misincorporation of this nucleotide opposite template adenine or the incorporation of it opposite template cytosine. The resulting A:GO and C:GO mispairs would be substrates for MutY and MutM glycosylases. If MutY removes A from A:GO mispair, this pathway will result in base substitution.
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wild-type \( P. \) putida\(^{198} \). The activity of MutY was shown to be important also in avoidance of mutations accumulating in starving cells of \( P. \) putida\(^{198} \).

Methyl-directed mismatch repair (MMR) pathway corrects base–base and insertion/deletion mismatches that have escaped the proofreading function of replicative DNA polymerase or are produced by error-prone DNA polymerases\(^{80, 131} \) (Figure 5). The MutS protein recognises and binds to mismatches\(^{222} \). In \( E. \) coli, together with MutL and in the presence of ATP, the MutS activates a third MMR protein, the MutH endonuclease\(^{7, 73} \). MutH cleaves the transiently unmethylated daughter strand at hemimethylated GATC sequences. Various exonucleases and the UvrD helicase complete the excision of the target strand. The excision step leaves long gaps, up to several thousand bases, which are repaired by DNA polymerase pol III and DNA ligase\(^{131} \). Although the genes encoding MutS and MutL homologues have been found in a wide diversity of bacteria, only close relatives of \( E. \) coli encode MutH homologues\(^{55} \). Based on the relationship between MutH and the restriction enzyme \( \text{Sau}3\)AI and the structural similarity to the other type II restriction enzymes\(^9 \) it is reasonable to suppose that MutH analogues may have evolved from different restriction systems. 

\( P. \) aeruginosa species also lack the \( \text{mutH} \) homologue. The other genes for MMR pathway (\( \text{mutS}, \text{mutL} \), and \( \text{uvrD} \)) have been recently isolated and characterised in \( P. \) aeruginosa\(^{164} \). Interestingly, despite a high homology to the corresponding \( E. \) coli sequences, none of the MMR genes of \( P. \) aeruginosa PAO1 complemented the increased mutation frequencies of \( E. \) coli strains defective for \( \text{mutS}, \text{mutL}, \) or \( \text{uvrD} \)^\(^{163} \) (Table 9). This suggests that complex interactions required between MMR enzymes and other proteins may be species-specific. The \( \text{mutS} \) gene of \( P. \) putida characterised in\(^{112} \) was unusual due to the lack of the sequence encoding approximately 300 amino acids from the N-terminal part of MutS. However, analysis of complete genome sequence of \( P. \) putida strain KT2440 demonstrates that this strain has a full-length \( \text{mutS} \) gene.

Nucleotide excision repair (NER) removes a wide variety of lesions from bacterial genomes through a set of coordinated reactions carried out by multiprotein complex, the UvrABC endonuclease\(^{196} \) (Figure 6). The UvrD helicase (which participates also in MMR pathway), in concert with DNA polymerase I, removes the damaged 12-13-nt oligonucleotide. Then a repair patch is synthesised by pol I, and sealed by DNA ligase. Characterisation of \( \text{uvrD} \) mutants of \( P. \) aeruginosa have revealed three amino acid substitutions within the conserved ATP binding site of the deduced UvrD polypeptide that abolished MMR activity but did not affect UvrABC-mediated excision repair\(^{163} \). This is the first published report indicating that the activities of UvrD in NER and MMR might be different (Table 9).

The expression of certain DNA repair systems may be differently controlled in \( E. \) coli and in \( P. \) aeruginosa\(^{164} \) (Table 9). For example, in \( E. \) coli the \( \text{uvrA} \) and \( \text{uvrB} \) genes are members of the SOS regulon and are induced by DNA damage, whereas the \( \text{uvrC} \) is constitutive\(^{64} \). In contrast, the \( \text{uvrB} \) homologue of
Figure 5. MMR pathway in E. coli. The first step is the detection of a mismatch through the binding of MutS dimer to heteroduplex DNA. Only one of the two subunits contacts the mismatch. The strand discrimination signal in the E. coli MMR system is provided by the transiently unmethylated state of newly synthesised DNA. MutH protein cleaves the unmethylated strand of a hemimethylated dam methylation site, thereby marking the nicked strand for exonucleolytic removal and resynthesis. MutH endonucleolytic activity is activated by a complex of MutS, MutL, and mismatched DNA, and requires ATP hydrolysis. MutH can nick DNA on either side of a mismatch. The activity of UvrD helicase (DNA helicase II) is needed to unwind the duplex DNA molecule. MutL also directly interacts with UvrD (this interaction is not shown in the figure). MutL is speculated to load the UvrD helicase onto the nick in a directional manner so that DNA unwinding proceeds toward the mismatch. The single-stranded template strand is protected by a single-strand binding protein SSB. Resynthesis is performed by DNA polymerase III and repair is completed by ligation of the remaining nick by ligase. Both the translocation model and the sliding clamp model (see, e.g., ref. [131] for a review) assume that MutS is released from the mismatch. Alternatively, results in ref. [91] suggest that MutS loading onto a mismatch induces the formation of a higher order complex containing multiple MutS homodimers, which can productively interact with MutL in ATP-hydrolysing conditions and generate a specific ternary complex, which might communicate with MutH. The growth of a MutS–DNA complex at the mismatch is illustrated only on one side of the mismatch.
Figure 6. NER pathway in *E. coli*. UvrA2B complex scans DNA until its movement is inhibited by the presence of bulky base damage. Initial damage recognition results in conformational changes in the UvrA2B–DNA complex, so that UvrB binds specifically at the damaged site, and UvrA is replaced by UvrC. The binding of UvrC to UvrB causes UvrB to cut the DNA at the fourth or fifth phosphodiester bond 3' to the lesion. The UvrC protein then cuts DNA at the eighth phosphodiester bond 5' to the lesion. Once the DNA is cut, the UvrD helicase removes 12-13-nt oligonucleotide containing the lesion, and DNA polymerase I resynthesises the strand that was removed. Finally, the ligase seals the single-strand nick.

*P. aeruginosa* PAO1 was not inducible by DNA damage\(^{188}\). Localisation of the promoter of the *P. aeruginosa* uvrB gene revealed that it lacked the LexA-binding sequence and was expressed constitutively.

Formation of a pyrimidine dimer is a common type of DNA lesion caused by UV irradiation, and can be reversed by a special type of DNA repair called photoreactivation\(^{107}\). Photoreactivation is unique among DNA repair systems since it involves in direct reversal of the UV-induced lesion: It involves a single enzyme called photolyase which binds a pyrimidine dimer and, in a light-dependent step, monomerizes the dimer and thereafter
dissociates from the repaired lesion\textsuperscript{107}. The gene encoding photolyase (\textit{phr}) isolated from \textit{P. aeruginosa} was used to construct insertional \textit{phr} mutants of \textit{P. aeruginosa} and \textit{P. syringae}\textsuperscript{106}. Analyses of the UV-B survival of these mutants demonstrated that under photoreactivation conditions other mechanisms such as NER and the action of \textit{rulAB}-encoded pol V-like enzyme were also active contributors to cell survival. The fact that the expression of the \textit{rulAB}-encoded protein increased the survival of \textit{P. aeruginosa} \textit{phr uvrA} double mutant by over 23,000-fold illustrates the importance of this enzyme whenever other DNA repair systems have not been able to remove the DNA replication-blocking damage.

6.4. Heritable Mutators in \textit{Pseudomonas}

The efficiency of DNA repair in a cell is an important mechanism controlling the frequency of mutations. In many cases, a mutator phenotype is induced by inactivation of genes that code for DNA repair enzymes\textsuperscript{142}. The most potent mutator strains in \textit{E. coli} are those lacking either the DNA polymerase III proofreading subunit (\textit{mutD}) or the methyl-directed mismatch repair system (\textit{mutS}, \textit{mutL}, \textit{mutH}). The \textit{mutM mutY} double mutant of \textit{E. coli} defective for DNA repair system that corrects mistakes occurring due to the oxidation of guanine (GO repair) is as strong a mutator as \textit{mutD}, and about an order of magnitude stronger than a strain lacking \textit{mutS}\textsuperscript{141}.

Most mutations are likely to be deleterious, and so the spontaneous mutation rate is generally held at a low level\textsuperscript{49}. However, a proportion of clones from natural populations of pathogenic and commensal bacteria have a strong mutator phenotype\textsuperscript{117, 134, 164}. By selecting a favourable allele, natural selection indirectly selects the DNA in which this mutation occurred. Consequently, mutator alleles can spread in microbial populations by hitchhiking: mutators can acquire favourable alleles more frequently than non-mutators and will therefore increase in frequency if the advantage of beneficial alleles is greater than the cost of being a mutator\textsuperscript{230}. Spread of mutator alleles has been documented in \textit{P. aeruginosa} populations infecting cystic fibrosis (CF) patients. In the study of spontaneous mutation rates of \textit{P. aeruginosa} isolates from chronically infected CF patients, Oliver et al.\textsuperscript{164} found that the lungs of 11 out of the 30 CF patients were colonised by mutator strains. In contrast, a mutator phenotype was not found in 75 non-CF patients acutely infected with \textit{P. aeruginosa}. The bacterial populations chronically infecting the lungs of CF patients have to adapt to the highly compartmentalised and anatomically deteriorating lung environment, as well to the challenges of the immune defence and antibiotic therapy. Hence, severe population bottlenecks occurring after strong selective challenges enrich mutators within a population.
Subsequent studies by complementation\textsuperscript{164} have revealed that seven out of eleven independent mutators studied were defective in the MMR system. Four carried mutations in \textit{mutS}, two in \textit{mutL}, and one in \textit{uvrD}.

Some mechanisms facilitating the acquisition of a mutator phenotype may have been selected through evolution\textsuperscript{175}. Rocha et al.\textsuperscript{189} hypothesise that inactivation of the MMR pathway might be positively selected. This argument is based on results of DNA sequence analysis indicating that the \textit{mutS} and \textit{mutL} sequences, in comparison with 1,000 random genes, contain larger number of direct repeats potentially capable of inducing phenotypic variability by generating deletions. They also suggest that results of the analysis are consistent with experimental evidence showing that deletions in the \textit{mutS} gene are a major source of mutator phenotypes in natural isolates. The existence of a possible recombinational hot spot inside the \textit{P. aeruginosa mutS} gene has also been suggested in Ref. 163. Note that the MMR not only protects against replication errors but also acts as a barrier to the recombination between moderately divergent DNA sequences. MMR-deficient cells carry out homologous recombination resulting from interspecies crosses three orders of magnitude more frequently than wild type cells\textsuperscript{181, 217}. Ochman \textit{et al.}\textsuperscript{159} have suggested that lateral transfer and recombinational reshuffling have played an important role in the generation of microbial diversity.

7. MUTAGENESIS IN \textit{PSEUDOMONAS} UNDER ENVIRONMENTAL STRESS

In a growth-restricting environment (e.g., during starvation, host invasion by a pathogen), mutants arise that are able to take over the population by a process known as stationary-phase mutation\textsuperscript{60}. Many examples of stationary-phase mutagenesis in bacteria are referred in ref. [60]. Although most of the research on stationary-phase mutation has exploited \textit{Escherichia coli}-based model systems, similar observations have also been made in other organisms including \textit{Pseudomonas} species (many examples are listed in Table 8). One characteristic feature of stationary-phase mutations is their specific spectrum, different from that occurring among the mutants of actively growing bacteria\textsuperscript{61, 96, 173, 193}. This has suggested that stationary-phase mutations occur via different molecular mechanisms than mutations which arise during growth\textsuperscript{60}. Some studies suggest that starvation conditions encountered during stationary-phase incubation may permit a transient increase in the mutation rate due to variety of factors, including decreased fidelity of DNA replication and reduction of DNA repair activity\textsuperscript{19, 59, 213, 238}. Roth \textit{et al.}\textsuperscript{194} have proposed an alternative model that explains the occurrence of stationary-phase mutations
without requiring regulated mutability. However, SOS induction has been shown to occur spontaneously also in static bacterial populations\(^229\). Error-prone DNA polymerases pol IV, and pol V in \textit{E. coli} are induced as part of the SOS regulon in response to DNA damage\(^272\). Pol IV and pol V are involved in stationary-phase mutagenesis in \textit{E. coli}\(^12, 25, 139, 263\). Bjedov \textit{et al.}\(^14\) have demonstrated that natural isolates of \textit{E. coli} exhibit increased mutation rates under stress encountered in aging colonies. The stress-inducible mutagenesis is genetically controlled by RpoS and the carbon-sensing regulators CyaA and CRP. Results presented in ref. [14] support the hypothesis that stress-induced mutagenesis is genetically programmed evolutionary strategy.

In addition to processes that are responsible for the generation of point mutations, the increase in transposition frequency in starving bacteria also gives a chance to generate genetic variation in a microbial population. There is evidence that starvation is accompanied by increased transposition frequency of many mobile elements\(^78, 96, 113, 146, 206\). Naas \textit{et al.}\(^149\) showed that a large number of IS-related rearrangements have occurred in a 30-year-old stab, leading to a highly polymorphic population of cells. Studies of glucose-limited chemostat cultures have demonstrated that Tn5 and Tn10 can increase fitness of \textit{E. coli}\(^13, 32\) whereas the fitness effect of Tn10 was associated with transposition of IS10 into new sites in the genome\(^31, 32\). Upregulation of transposition of transposon Tn4652 by the host factors IHF and RpoS\(^89\) is one of the interesting examples illustrating the involvement of transposition of mobile DNA elements in stationary-phase mutagenesis in \textit{Pseudomonas}. Many other transpositional events discussed above in ‘Involvement of transposition in mutagenesis’ could also be ascribed to mechanism of stationary-phase mutations (Table 8). Interestingly, several IS-elements and transposons carry outward-directed promoters or part of promoter sequences (e.g., the \(-35\) hexamer) that can activate silent genes\(^66, 128\). In many cases, these promoter sequences have no obvious functions related to the regulation of genes internal to the element. Therefore, one should speculate that the presence of outward-directed promoters at the ends of many transposable elements hints that some of these elements may have been evolved to generate genetic variations.

Evidence supporting the idea that different mechanisms are responsible for the appearance of mutations in exponentially growing and stationary-phase cells has been found in studies using a \textit{P. putida} test system. This test system based on promoterless phenol degradation genes \textit{pheBA} as a reporter enabled to isolate and characterise the mutations (point mutations as well as insertions of transposable elements) accumulating in starving \textit{P. putida}\(^96\). The accumulation rate of Phe\(^+\) mutations on selective plates was found to depend on the physiological state of bacteria before the plating: The accumulation was much higher for bacteria plated from a stationary-phase culture than for those plated
from a growing culture\textsuperscript{96}. Moreover, the results of a recently published study\textsuperscript{198} suggest that mutation processes in cells that have been starving for a short period are not entirely compatible with those from a prolonged starvation. It appeared that the spectrum of stationary-phase mutations among early-arising mutants differed from that of late-arising ones. The occurrence of mutations, which started to increase later (e.g., 2- to 3-bp deletions), was dependent on stationary-phase sigma factor RpoS\textsuperscript{198}.

The possible involvement of error-prone DNA polymerase in stationary-phase mutagenesis in \textit{Pseudomonas} can be considered in ref. [138]. The UV response genes cloned from CAM-OCT plasmid enhanced the accumulation rate of \textit{P. putida} catabolic mutants\textsuperscript{138}. Therefore, it is likely that the ability of cells to expand the growth substrate range may be mediated by pol V-like function encoded on CAM-OCT plasmid. This chapter also demonstrated that the process of stationary-phase mutagenesis could be exploited to isolate the variants with potential industrial use by selecting new or improved catabolic activities in \textit{Pseudomonas} strains. Occurrence of pol IV-dependent mutations can be detected in \textit{P. putida} cells that have been starved several days (but not earlier) for carbon sources, and this mutagenic process is RecA-independent (R. Tegova, A. Tover, K. Tarassova, M. Tark, and M. Kivisaar, unpublished data).

The generation of strains with enhanced degradative potential in natural environment via stationary-phase mutagenesis has been demonstrated in the article by Sarand \textit{et al.}\textsuperscript{197}. These authors provided evidence that \textit{Pseudomonas} sp. strain CF600 can rapidly adapt its suboptimal ability to degrade 4-methylphenol in soil through enhancement of the ability of the pathway regulator DmpR to respond to 4-methylphenol.

Hospital isolates frequently present multidrug-resistance phenotypes as a consequence of constant selective pressure of antibiotics. The wild type \textit{P. aeruginosa} PAO1 expresses a low level of the antibiotic efflux pump, MexAB-OprM, which gives the bacterium natural resistance to a broad spectrum of antibiotics\textsuperscript{172}. Results presented in ref. [3] indicated that the emergence of multidrug-resistant mutants in \textit{P. aeruginosa} increases under antibiotic challenge. The authors demonstrated that the incubation in the presence of tetracycline favoured the emergence of multidrug-resistance mutants expressing the efflux pump of antibiotics constitutively. Based on calculations of the number of mutants accumulating per day per viable cells in the population, the authors concluded that the mutation rate of tetracycline resistance increased by $10^{5}$ times after four days of incubation under tetracycline challenge.

Phenotypic switching is one of the strategies of micro-organisms for coping with environmental changes, and it is usually caused by high-frequency DNA rearrangements\textsuperscript{53}. In natural habitats, bacteria are living in a structured environment: They usually grow as biofilms, organised communities of cells embedded in an extracellular polysaccharide matrix and attached to a surface\textsuperscript{58}. 

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Pseudomonads are known for their striking ability to adapt to various ecological niches. *P. fluorescens* is one of the model organisms chosen for experimental studies of adaptive evolution in bacteria. This organism has been employed in studies of the ecological and genetic causes of phenotypic divergence in microcosm populations. When selected in a spatially structured environment, populations of *P. fluorescens* rapidly diverge, producing a range of morphologically distinct niche specialist genotypes that are maintained by negative frequency-dependent selection. The radiation is driven by competition among the niche specialists. While no morphological variation can be detected in a homogeneous environment, these experiments clearly demonstrate that the phenotypic divergence is dependent upon ecological opportunity. The mutational origins of *P. fluorescens* phenotypic variants are still unknown. However, studies by Spiers *et al.* have indicated that one set of the mutants forming wrinkled colonies may be caused by a mutation that alters the cell cycle regulation of cellulose-like polymer production, causing this polymer to be overproduced. The rapid adaptation to different environmental niches has been also observed in *P. aeruginosa* biofilm formation. The readily visible phenotypes of colony morphology mutants have been shown to be associated with mutations in regulators. Therefore, it is possible that mutations occurring in regulatory pathways may play a general role in phenotypic switching and subsequent bacterial diversification in a structured environment.

Evidence exists that environmental signals can specifically control the rate of phenotyping switching in *Pseudomonas* species. It has been shown that *P. aeruginosa* in the sputum of CF patients exists primarily as a biofilm. Studies by Drenkard and Ausuble have demonstrated that antibiotic-resistance phenotypic variants of *P. aeruginosa* with enhanced ability to form biofilms arise at high frequency both in vitro and in the lungs of CF patients. These variants reverted at high frequency to wild type in the absence of antibiotic in the environment. The phenotypic switching between the antibiotic-resistant and antibiotic-susceptible forms of *P. aeruginosa* in CF patients was controlled by the activity of two-component response regulator PvrR. The active PvrR repressed the emergence of antibiotic resistant variants and biofilm formation, whereas a mutated pvrR increased the frequency of appearance of resistant variants with respect to the wild type. The proportion of the population that became resistant to antibiotics through phenotypic variation was dependent on environmental conditions: The increase in the frequency of appearance of resistance variants occurred at a lower temperature, in the presence of NaCl, and under starvation. Phenotypic switching in *P. tolaasii* was also modulated by an environmentally responsive regulatory factor. DNA rearrangements associated with phenotypic switching between the pathogenic smooth and non-pathogenic rough forms of *P. tolaasii* occurred due to the reversible duplication of a 661-bp element within the sensor protein-encoding *pheN* locus in a RecA-dependent manner.
Some pathogenic bacteria have evolved highly mutabile ‘contingency loci’ that can rapidly change due to the presence of DNA sequence repeats. Hypermutable genes of pathogens encode cell surface molecules, such as adhesins or invasins involved in interactions with host molecules. Analysis of the P. aeruginosa oprD mutants (lacking a specialised porin protein whose inactivation causes specific antibiotic resistance) from clinical and environmental isolates indicated that similar mechanisms could play a role in the emergence of new alleles in Pseudomonas species as well. Whether the loci responsible for phenotypic switching in biofilm formation can also contain mutational hot spots is still unknown.

8. CONCLUSIONS

In nature, microbes are constantly confronted by variable and stressful environments. Transposition and other mutational processes have important roles in the genetic adaptation of bacteria under environmental stress (Table 8). Bacteria from the genus Pseudomonas are known for their ability to colonise multiple habitats and to adapt rapidly to a new environment. The involvement of transposable elements in acquisition and recruitment of genetic material in Pseudomonas is well documented. Also, a number of publications demonstrate that transposition can cause genetic rearrangements and affect gene activity. Some recent studies have revealed that despite its potential deleterious effect to the host cell, transposition may be up-regulated under certain environmental conditions (e.g., starvation). Other mutations occurring in stressed cells are stimulated by mechanisms that possibly involve increased mutation rates (induction of error-prone DNA polymerases, inefficiency of DNA repair systems). Although systematic studies of mutagenic processes in Pseudomonas are still awaiting, several new aspects have been discovered in this group of bacteria (summarised in Table 9). Also, recent papers have provided evidence that environmental signals can specifically control the rate of phenotypic switching in Pseudomonas species. Therefore, it is reasonable to expect that the coming years will widen our awareness of physiological regulation of mutagenic processes in bacteria.

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