Modulation of gene expression through chromosomal positioning in *Escherichia coli*

Carolina Sousa,† Víctor de Lorenzo and Angel Cebolla†

Variations in expression of the *nah* genes of the NAH7 (naphthalene biodegradation) plasmid of *Pseudomonas putida* when placed in different chromosomal locations in *Escherichia coli* have been studied by employing a collection of hybrid mini-Tn5 transposons bearing lacZ fusions to the Psal promoter, along with the cognate regulatory gene *nahR*. Insertions of Psal–lacZ reporters in the proximity of the chromosomal origin of replication, oriC, increased accumulation of β-galactosidase in vivo. Position-dependent changes in expression of the reporter product could not be associated with local variations of the supercoiling in the DNA region, as revealed by probing the chromosome with mobile gyrB–lacZ elements. Such variations in β-galactosidase activity (and, therefore, the expression of catabolic genes) seemed, instead, to be linked to the increase in gene dosage associated with regions close to oriC, and not to local variations in chromosome structure. The tolerance of strains to the selection markers borne by the transposons also varied in parallel with the changes in LacZ levels. The role of chromosomal positioning as a mechanism for the outcome of adaptation phenotypes is discussed.

**Keywords**: biodegradation, catabolic operons, positional effects, NahR, gyrB

**INTRODUCTION**

Our laboratory is interested in the mechanisms by which Gram-negative bacteria, in particular *Pseudomonas*, evolve the ability to employ recalcitrant hydrocarbons as carbon sources (de Lorenzo & Pérez-Martin, 1996; van der Meer et al., 1992). A critical step in the success of a new pathway is the acquisition of an adequate expression control system that is responsive to pathway substrates or related metabolic intermediates (de Lorenzo & Pérez-Martin, 1996). Generally, promoters are controlled by specific regulators that trigger transcription initiation when cells face a certain chemical or physical signal. However, the effective level of expression of a particular gene or operon is subject to the general physiological status of the cells. Such status selects a window of expression for the sake of an adequate energetic return. General mechanisms that influence gene expression in *vitro* include histone-like proteins (Nash, 1996), superhelical density (Liu & Wang, 1987; Menzel & Gellert, 1983) and DNA methylation (Plasterk et al., 1983). These may differ depending on the physiological status or even, as has been suggested, on the chromosomal location (Schmid & Roth, 1987). This last possibility is particularly appealing, since catabolic operons appear frequently within transposons (Wyndham et al., 1994) and could, therefore, employ differential positioning within a replicon as a mechanism for fine-tuning an optimal level of expression.

During the course of previous studies (de Lorenzo et al., 1993), we had noticed that the activity of three different catabolic systems of *Pseudomonas putida* varied significantly depending on their position in the bacterial chromosome, as revealed through lacZ fusions to relevant promoters. These were: (i) the *Pu* promoter of the TOL (toluene biodegradation) plasmid pWW0, (ii) the *Pm* promoter of the TOL plasmid and (iii) the Psal promoter of the NAH7 (naphthalene biodegradation) plasmid. Since very minor variations in the expression of adaptation genes have a major influence on the long-term outcome of a particular strain (Elena et al., 1996; Negri et al., 1994), we were interested in exploring chromosomal positioning as a factor influencing gene
METHODS

Strains, media and general procedures. Bacteria and plasmids used in this work are listed in Table 1. Cells were grown in Luria broth (LB) or M9-glucose (0.2\%) minimal medium (Maniatis et al., 1982) supplemented, where appropriate, with 50 \( \mu \)g rifampicin (Rif) ml\(^{-1} \), 50 \( \mu \)g kanamycin (Km) ml\(^{-1} \), 150 \( \mu \)g ampicillin (Ap) ml\(^{-1} \), 25–50 \( \mu \)g streptomycin (Sm) ml\(^{-1} \) or 50 \( \mu \)g X-Gal ml\(^{-1} \). DNA manipulations, including Southern blotting of agarose gels, were carried out according to Washington (1989). B-Galactosidase (\( \beta \)-Gal) accumulation was determined with the sequence \( \text{PgyrB}::\text{galT}^-\text{galK}^-\text{lacZ} \) (in essence, a transcriptional \( \text{PgyrB}::\text{lacZ} \) fusion) as a 7.8 kb NotI fragment. This segment was cloned into the transposon delivery plasmid pUT/mini-Tn5Km2 (de Lorenzo et al., 1990) to generate pTgyrB. This plasmid contains a hybrid mini-transposon in which the \( \text{PgyrB}::\text{lacZ} \) fusion is flanked by Km and Sm interposons that shield the reporter system from any read-through transcription from nearby promoters after chromosomal integration. The organization of the resulting supercoiling-responsive probe is shown in Fig. 1.

Mobilization and transposition. Insertion of hybrid mini-transposons into the chromosome of the K-12 \( \text{E. coli} \) strain W3110 was done following the procedure described in detail by de Lorenzo & Timmis (1994). Matings between the donor strain \( \text{E. coli} \) S17-1lipir, transformed with the delivery plasmid indicated in each case, and the recipient strain were run at 30 \(^\circ\)C for 2–4 h on the surface of a filter placed on an LB plate supplemented with 1\% sodium citrate. The cells were then incubated at 30 \(^\circ\)C for 2–4 h on the surface of a filter placed on an LB plate supplemented with 1\% sodium citrate. The cells were then

### Table 1. Bacteria and plasmids

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant genotype/phenotype/characteristics</th>
<th>Reference/origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{E. coli} ) K12</td>
<td>( \Delta(\text{ara-leu}), \text{araD} , \text{lacX} , 74 , \text{galE} , \text{galK} , \text{phoA} , \text{thi-1} )</td>
<td>Herrera et al. (1990)</td>
</tr>
<tr>
<td>CC118</td>
<td>( \text{rpsE} , \text{rpoB} , \text{argE} , \text{Am} , \text{recA} )</td>
<td>Herrera et al. (1990)</td>
</tr>
<tr>
<td>CC118lipir</td>
<td>CC118 lysogenized with lipir phage</td>
<td>Herrera et al. (1990)</td>
</tr>
<tr>
<td>S17-lipir</td>
<td>( \text{T}^+ , \text{Sm}^- , \text{recA} , \text{thi-1} )</td>
<td>de Lorenzo &amp; Timmis (1994)</td>
</tr>
<tr>
<td>W3110lac</td>
<td>( \text{hyA} , \text{deoC} , \text{F}^- , \text{INV2D-rnmE} , \Delta\text{lac} )</td>
<td>Daniels (1990)</td>
</tr>
<tr>
<td>pUT</td>
<td>( \text{Ap}^+, \text{trp}^-::\text{lacZ} ) promoter probe vector</td>
<td>de Lorenzo et al. (1990)</td>
</tr>
<tr>
<td>pUS</td>
<td>( \text{Ap}^+, \text{lacZ} ) promoter probe vector</td>
<td>de Lorenzo et al. (1990)</td>
</tr>
<tr>
<td>pCNB4</td>
<td>( \text{Ap}^+, \text{Km}^+, \text{oriK} ) mobR4, delivery vector for mini-Tn5 \text{Km} \text{Psal}</td>
<td>de Lorenzo et al. (1993)</td>
</tr>
<tr>
<td>pCNB4-lacZ</td>
<td>Same as pCNB4, but inserted with a promoterless ( \text{trp}^-::\text{lacZ} ) reporter downstream of the Psal promoter</td>
<td>de Lorenzo et al. (1993)</td>
</tr>
<tr>
<td>pGP704</td>
<td>( \text{Ap}^+, \text{oriK} ) mobR4 M13 tg131 polylinker</td>
<td>Miller &amp; Mekalanos (1988)</td>
</tr>
<tr>
<td>pUT</td>
<td>( \text{Ap}^+, \text{trp} ) gene of Tn5-III50, inserted in SalI site of pGP704</td>
<td>Herrera et al. (1990)</td>
</tr>
<tr>
<td>pUT/mini-Tn5 Km2</td>
<td>( \text{Ap}^+, \text{Km}^+, \text{R6KoriV} , \text{RP4oriT} , \text{mini-Tn5} , \text{Km2} ) transposon-vector delivery plasmid</td>
<td>de Lorenzo et al. (1990)</td>
</tr>
<tr>
<td>pIC548</td>
<td>( \text{Ap}^+, \text{Sm}^-, \text{PgyrB}::\text{galK}^-\text{lacZ} )</td>
<td>Macián et al. (1994)</td>
</tr>
<tr>
<td>pUPO</td>
<td>( \text{Ap}^+, \text{aadA} ) promoter inserted as a 1-1 kb ( \text{BamHI-BclI} ) insert in pU78</td>
<td>This work</td>
</tr>
<tr>
<td>pUgyrB</td>
<td>( \text{Ap}^+, \text{PgyrB} ) derivative bearing a ( \text{PgyrB}::\text{galK}^-\text{lacZ-Sm}^- ) cassette as a NotI insert</td>
<td>This work</td>
</tr>
<tr>
<td>pTgyrB</td>
<td>( \text{Ap}^+, \text{Km}^+, \text{PgyrB} ) derivative vector for mini-Tn5 ( \text{PgyrB}::\text{lacZ} )</td>
<td>This work</td>
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The catabolic NAH system and organization of Psal–lacZ and Pgyr–lacZ transposons. (a) Regulatory features of the naphthalene-degrading pathway of the NAH7 plasmid of P. putida. Upon exposure of the cells to naphthalene vapour, the basal expression of the upper operon is enough to generate a certain amount of intracellular salicylate. This intermediate and transcriptional terminator at the end of the naphthalene-degrading pathway of the NAH7 plasmid of P. putida strain KT2442 (de Lorenzo et al., 1993). This behaviour could not be attributed to readthrough transcription from nearby external promoters, since design of the lacZ-bearing mini-transposons employed included strong terminators flanking the reporter system. We therefore examined the basis of such differential expression. For this, we used the salicylate-responsive Psal promoter of the NAH7 plasmid and transferred the regulatory elements into E. coli strain KT2442 (de Lorenzo et al., 1993). This behaviour could not be attributed to readthrough transcription from nearby external promoters, since design of the lacZ-bearing mini-transposons employed included strong terminators flanking the reporter system. We therefore examined the basis of such differential expression. For this, we used the salicylate-responsive Psal promoter of the NAH7 plasmid and transferred the regulatory elements into E. coli strain W3110 in order to have a more defined background for genetic analyses.

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RESULTS AND DISCUSSION

Chromosomal location modulates expression of nah genes of the NAH7 plasmid

This study was initiated by our earlier observation that independent insertions of hybrid mini-transposons bearing lacZ fusions to the Pu, Pm and Psal promoters of catabolic plasmids TOL and NAH of P. putida gave rise to different levels of accumulation of β-Gal in response to the pathway inducers m-xylene, m-toluate and salicylate, respectively, when randomly inserted into the chromosome of P. putida strain KT2442 (de Lorenzo et al., 1993). This behaviour could not be attributed to readthrough transcription from nearby external promoters, since design of the lacZ-bearing mini-transposons employed included strong terminators flanking the reporter system. We therefore examined the basis of such differential expression. For this, we used the salicylate-responsive Psal promoter of the NAH7 plasmid and transferred the regulatory elements into E. coli strain W3110 in order to have a more defined background for genetic analyses.

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Fig. 2. Phenotypes produced by different insertions of mini-Tn5 nahR/Psal-lacZ. Eight E. coli W3110 exconjugants bearing independent insertions of the mini-transposon were examined for (a) their ability to accumulate β-Gal in the presence or absence of the Psal inducer, salicylate, and (b) their growth on media with increasing concentrations of Km, (a Km-resistance marker accompanies the lac2 fusion within the transposon; see Fig. 1). The locations of the insertions in distinct chromosomal AvrII restriction fragments are indicated at the bottom.

shown in Fig. 1, the mobile element contained a DNA segment that included the gene for the salicylate-responsive NahR regulator (transcribed through its native promoter, Pr) along with the cognate Psal promoter in front of a lac2 reporter gene. This segment was flanked upstream by an Δ-Km element and downstream by a T7 terminator, which isolated the reporter system from external transcription sources. The mating E. coli S17-1pir(pCNB4-lacZ) × E. coli W3110 gave rise to Ap⁰ Km⁻ colonies with a frequency of 10⁻⁶, out of which eight exconjugants were taken for further analysis. As shown in Fig. 2(a), all exconjugants accumulated β-Gal when induced with salicylate, thus indicating that the NahR/Psal system was functional in every case. However, the induction range did change two- to threefold depending on the insertion. Interestingly, we observed that the MIC of Km for each of the strains appeared to vary in parallel with the β-Gal accumulation driven by the Psal promoter (Fig. 2b). The position of each of the insertions in the E. coli W3110 chromosome was determined by physical means (Wong & McClelland, 1992), exploiting the presence of the unusual AvrII site within the mini-transposon (Fig. 1). This allowed us to match the insertions of the transposons within the AvrII restriction map of E. coli W3110 by making use of both published data (Daniels, 1990) and Southern blotting of the PFGE agarose gels with known probes (not shown). A typical gel used for mapping insertions is shown in Fig. 3. This analysis confirmed that the eight insertions were independent and were located in different portions of the E. coli chromosome. Very similar results were obtained when mini-transposons bearing lacZ fusions to the Pu or the Pm promoters of the TOL plasmid were used instead of mini-Tn5 nahR/Psal-lacZ (not shown), thus suggesting that variation in expression dependent on chromosomal location is a general phenomenon.

Probing supercoiling with a PgyrB-lacZ transposon

A simple explanation for the variations observed in the activity of the Psal-lacZ fusions, when inserted in independent chromosomal locations, is that the transposon could have inserted in regions with differing DNA topology. To examine this possibility, we constructed a specialized genetic probe, mini-Tn5 PgyrB-lacZ, which bears a lacZ transcriptional fusion with the promoter of the gene of the DNA gyrase B subunit. PgyrB promoter activity is induced when negative supercoiling decreases (Menzel & Gellert, 1983). The rationale of its utilization is that lacZ activity caused by the mini-transposon is predicted to be higher when inserted in more relaxed locations, while it should be lower in regions with higher superhelicity. Before going ahead with the random
transposition of mini-Tn5 PgyrB-lacZ, the behaviour of the gyrB promoter was first verified in the precursor plasmid pUgyrB (Table 1). As shown in Fig. 4, addition of 10 μg ml⁻¹ of the gyrase inhibitor coumermycin A₁ to cultures of E. coli W3110 bearing pUgyrB increased β-Gal accumulation twofold. In contrast, high osmolarity (NaCl 0.5 M) decreased β-Gal levels twofold. These differences matched the distribution of plasmid topoisomers extracted from cells grown in different conditions, as examined in chloroquine/agarose gels (not shown). As a control, a lacZ fusion to the constitutive promoter of the streptomycin/spectinomycin-resistance gene (aadA) of the Ω-Sm interposon contained in plasmid pUPO (Table 1) was used; it remained insensitive to either coumermycin or salt (Fig. 4).

Insertions of mini-Tn5 PgyrB-lacZ were generated from the mating E. coli S17-1pir(pTgyrB) × E. coli W3110. Thirty-three independent exconjugants with the expected phenotype (Km° Sm°, blue colonies in X-Gal medium) were used for further analysis. The levels of β-Gal differed by two- to threefold (98–264 β-Gal units, Fig. 5a). Nine exconjugants displaying increasing levels of β-Gal were chosen for further examination. PFGE of the chromosomal DNA of each of the strains digested with AvaII (Fig. 3) confirmed that each strain had a single insertion of the transposon and that they were placed in different chromosomal locations. The same strains were then challenged with conditions that alter superhelicity. Should separate regions of the chromosome have different degrees of supercoiling, conditions known to affect DNA helical density would have different effects in each region. However, if the chromosome behaves as a whole cccDNA molecule with respect to supercoiling (Drlica, 1992; Worcel & Burgi, 1972), then all regions should respond similarly to the challenge. To test these predictions, we first subjected the nine strains bearing separate insertions of mini-Tn5 PgyrB-lacZ to a challenge with a subinhibitory concentration of coumermycin A₁, with the purpose of transiently relaxing the chromosomal DNA. As shown in Fig. 5(b), under these conditions all strains increased their accumulation of β-Gal to the same extent. In a second series of experiments, cultures of the same strains were challenged with an addition of up to 0.5 M NaCl, which is predicted to increase negative DNA supercoiling (see above). In this case, accumulation of the reporter product decreased to the same extent in all strains, although the relative differences between them were maintained (Fig. 5b). These results indicate that the distinct chromosomal locations at which the reporter gyrB-lacZ transposon was inserted were equally affected by changes in the supercoiling. These results further support the hypothesis that overall chromosomal helicity is under a global control, and that domains with a different degree of supercoiling do not exist as such (Miller & Simons, 1993).
Variations in promoter activity depend on the
distance from the chromosomal replication origin

In order to examine the cause of the differential expression of the Psal-lacZ and the Pgyr-lacZ fusions, we mapped, with some accuracy, the position of each of the insertions selected. To this end, chromosomal DNA of each of the strains was digested not only with AvrII, but also with NotI (the mini-Tn5 transposons contain additional NotI sites; Fig. 1), so that two criteria could be used to locate the site of transposon insertion (Bachmann, 1990; Heath et al., 1992; Smith et al., 1987). Although some of the bands obtained in PFGE were not fully coincident with the published maps of *E. coli* W3110 (Daniels, 1990; Perkins et al., 1993), we could complete most of the mapping by performing Southern blot hybridizations of the PFGE gels with probes of known genes (Fig. 6). This allowed unequivocal assignment of the seven largest AvrII fragments, spanning >90% of the *E. coli* chromosome (Fig. 6). The remaining gaps in the map were irrelevant for our purposes. The results of the physical mapping are summarized in Fig. 6. Although the location of the insertions had a degree of error due to the low solution of the PFGE technique, we observed a correlation between expression of the lacZ fusions to PgyrB or Psal (see below) and the proximity to the chromosomal replication origin, oriC. The highest accumulation of β-Gal was observed in strain SG22, which bore a mini-Tn5 PgyrB-lacZ insertion in the 175 kb band (fragment G; Fig. 3), where the oriC is located. Strains SG01, SG27, SG33 and SG32, which accumulated β-Gal at levels significantly above the average, also carried insertions in restriction fragments relatively proximal to the oriC site. The lowest levels of β-Gal were observed in strains SG16, SG05, SG15 and SG30, which bore mini-Tn5 PgyrB-lacZ elements in the chromosomal portion spanning the replication termination site terC1A (band F) and the flanking fragments (bands A and E), respectively. These observations provide a key to understanding the basis of differential expression, since the proximity of the promoter to oriC might result in a transient increase of copy number during growth (Miller & Simons, 1993) due to initiation of rounds of bidirectional replication prior to the termination of preceding rounds. In view of this hypothesis, we re-examined the activity of the Psal-lacZ fusions generated through insertion of transposon mini-Tn5 napR/Psal-lacZ (see above) by mapping them within distinct chromosomal fragments. The data in Fig. 2(b) are consistent with the notion that proximity to the replication origin is the major cause of the differences between the β-Gal levels accumulated by the strains: *E. coli* strain W3110 bearing insertion NA8 (Fig. 2) and displaying the maximum Psal-lacZ activity, had the reporter element within the 175 kb fragment that contains oriC (G band). Mini-Tn5 napR/Psal-lacZ insertions NA1, NA18 and NA20, placed in sites proximal to the oriC locus (within fragments C and D, respectively), also displayed relatively high β-Gal levels. Conversely, the strains with the lowest activity (insertions NA4, NA11, NA24 and NA25) had their insertions at sites more proximal to the replication termination site terC (fragments B and E). It seems, therefore, that proximity to oriC is a general factor influencing gene expression, at least in cases of rapid growth (Miller & Simons, 1993; Pavitt & Higgins, 1993). Interestingly, such influence affects not only expression of dispensable activities (such as β-Gal), but can also determine gross phenotypes. As shown graphically in Fig. 2(b) and Fig. 6, the level of tolerance to Km or Sm afforded by insertion of either mini-Tn5 napR/Psal-lacZ (Km') or mini-Tn5 PgyrB-lacZ (Km' Sm') increased or decreased in parallel with the activity of the accompanying lacZ fusions. In these cases, proximity of the resistance genes to the

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**Fig. 6.** Location of selected mini-Tn5 PgyrB-lacZ insertions in the chromosome of *E. coli* W3110 as related to Sm-tolerance. The figure summarizes the positions of each of nine transposon insertions as mapped through PFGE of chromosomal DNA digested with AvrII (see Fig. 3). The MIC of Sm required to prevent growth of each strain is indicated. The AvrII digestion map of the *E. coli* W3110 chromosome is shown lined up with the genetic map. The positions of various gene probes (Beck & Bremer, 1980; Boullain et al., 1986; Braun-Brenton & Hofnung, 1986; Daniels, 1990; de Lorenzo et al., 1990; Hayward et al., 1991; Nakamura & Inouye, 1979; Wee et al., 1988; Williams et al., 1986) used as references to match the digestion products with the published *E. coli* W3110 map (Daniels, 1990) are also indicated. The location of the small DNA segments shown as filled black boxes are inferred from published data. Note maximum and minimum MICs around positions close to oriC and terC, respectively.
replication origin determined production of a phenotype required for survival.

Modulation of gene expression through chromosomal positioning?

There is increasing evidence that building of a higher-order chromosomal structure is basically a stochastic process (Higgins et al., 1996). Although barriers to supercoil diffusion exist (Worcel & Burgi, 1972), they seem to vary in position from cell to cell or within one cell over time (Higgins et al., 1996). On this basis, it seems that variable expression of differently positioned genes is unrelated to the responsiveness of the promoters to supercoiling. In fact, it seems that both strong and weak promoters are stochastically distributed throughout the entire chromosome (Chuang et al., 1993), which argues against the existence of defined silent or active chromosomal domains in bacteria. However, genes placed in the proximity of the replication origin are systematically expressed at higher levels (Miller & Simons, 1993; Pavitt & Higgins, 1993; this work). This may happen because of a transient increase in copy number during growth. Although the differences observed are relatively small, they may become significant for organisms adapting to a harsh environment (e.g. antibiotic resistance or growth on unusual carbon sources). At least in the case of antibiotics, it seems that minimal variations in MICs determine the outcome of resistant strains in the presence of subinhibitory concentrations of the selective agent (Negri et al., 1994). By the same token, it is possible that small variations in the expression of catabolic operons contribute to the success of a certain pathway in non-extreme selection conditions. In this context, our results suggest that chromosomal positioning might be one more mechanism of changing environments by modulating the degree of gene expression to an optimal window of activity.

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