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Genetics

Homology between nucleotide sequences of promoter regions of *nah* and *sal* operons of NAH7 plasmid of *Pseudomonas putida*

(S1 nuclease mapping/salicylate/*nahR*/transcription initiation)

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ABSTRACT **The *in vivo* transcription start sites of the *nah* and *sal* operons of the NAH7 plasmid were determined by S1 nuclease mapping and the nucleotide sequence surrounding these transcription start sites was determined. Since expression of both of these operons is coordinately controlled by the product of the transcriptional activator gene *nahR*, the sequences were compared to locate potential sites involved in common regulation. In the 100-base-pair region preceding transcription start sites of both operons, three regions of extensive homology were found and may be involved in *nahR*-mediated transcriptional control: (*i*) between −80 and −60 with 81% homology; (*ii*) between −40 and −28 with 75% homology; (*iii*) between −1 and +15 with 70% homology. Comparison of the promoter sequences of *nah* and *sal* with the analogous sequences of the *xylABC* and *xylDEFG* operons of the TOL plasmid showed little homology between the 5′ regions of these two sets of positively regulated hydrocarbon degradation operons. In addition, the transcription start site of the *nahR* regulatory gene was located and its promoter sequence was determined. The *nahR* promoter overlapped at the −35 position of the *sal* promoter; however, the *nahR* gene is transcribed in the opposite direction. Sequences similar to the consensus sequences of *Escherichia coli* promoters (at −35 and −10) were found in *nah*, *sal*, and *nahR* at the appropriate positions.**

The NAH7 plasmid is a member of a class of self-transmissable plasmids, predominantly found in *Pseudomonas*, that encode the ability to utilize various aromatic compounds as sole carbon and energy source (1, 2). Molecular genetic analysis of two of these plasmids (NAH7 and TOL) has indicated an analogous organization and regulation (3–6). The catabolic genes for metabolism of aromatic hydrocarbons on NAH7 and TOL are organized in two operons (3–5). On NAH7 one operon, *nah* (*nahA–F*), encodes enzymes for metabolism of naphthalene to salicylate, whereas the other operon, *sal* (*nahG–M*), encodes enzymes for the metabolism of salicylate to tricarboxylic acid cycle intermediates. An analogous situation is found for the TOL plasmid where one operon encodes for metabolism of toluene or xylene to an aromatic acid and the other operon encodes enzymes for the metabolism of the aromatic acid to tricarboxylic acid cycle intermediates. Both sets of operons are coordinately induced >20-fold; induction requires the presence of a specific *trans*-acting regulatory gene and the respective inducer (5, 7–9).

Recent genetic evidence suggests that the *nahR* regulatory gene of the NAH7 plasmid encodes a protein, which, upon binding the inducer, salicylate, activates transcription of both catabolic operons, *nah* and *sal* (9). The site where the *nahR* gene product exerts its induced stimulation of transcription is probably located in the region 5′ to the transcription start site of each operon and could be similar to an analogous sequence in the 5′ flanking region of the *xylABC* or *xylDEFG* operons of the TOL plasmid (10, 11). A comparison of the promoter sequences of these operons could provide insight into the mechanism and origin of positive control systems of the environmentally important hydrocarbon degradation plasmids in the genus *Pseudomonas*.

To address these questions I have located and sequenced the transcription start sites of the *nah* and *sal* operons of NAH7. Substantial sequence homology exists between the 5′ flanking regions of the transcription start sites of the *nah* and *sal* operons but little homology with the nucleotide sequences of *xylABC*, *xylDEFG*, or other positively controlled promoters is evident. The transcription start site of the *nahR* regulatory gene was found 60 base pairs (bp) upstream of the transcription start site of *sal*. The *sal* and *nahR* promoters overlap at −35 but are transcribed in opposite directions.

MATERIALS AND METHODS

Materials. Materials and their sources are as follows: T4 polynucleotide kinase, P-L Biochemicals; other DNA enzymes, Bethesda Research Laboratories or New England Biolabs; antibiotics, 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal), electrophoresis chemicals, and calf thymus DNA, Sigma; [γ-^{32}P]ATP, Amersham; medium materials, Difco.

Plasmids. Plasmid pDV50 was constructed by ligation of *Hin*dIII-digested pKGX505 DNA (12) with *Hin*dIII-digested pBR325 DNA (13), transformation of *Escherichia coli* C600, and selection of Cm^r (chloramphenicol-resistant) transformants on L agar containing 30 μg of chloramphenicol per ml. Cm^r transformants were analyzed for plasmid content and a plasmid containing the 2.4-kilobase-pair (kb) *Hin*dIII fragment of pKGX505 was isolated and designated pDV50 (Fig. 1).

Plasmid pSC3 was constructed by ligation of *Pst* I–*Eco*RI-digested pKGX530 DNA (12) with *Pst* I-digested pUC8 DNA (14). After transformation of *E. coli* JM83, Ap^r (ampicillin-resistant) transformants were selected on L agar containing 100 μg of ampicillin per ml and spread with 0.04 ml of 4% X-Gal. One white Ap^r transformant was shown to contain the 2.7-kb *Pst* I fragment of pKGX530 inserted in pUC8 and was designated pSC3 (Fig. 1).

Plasmid pSR1 was constructed by ligation of *Sal* I-digested pDV50 with *Sal* I-digested pUC8 DNA. White Ap^r transform-

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Abbreviations: kb, kilobase pair(s); bp, base pair(s); Cm^r, chloramphenicol-resistant; Ap^r, ampicillin-resistant; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; ORF, open reading frame.

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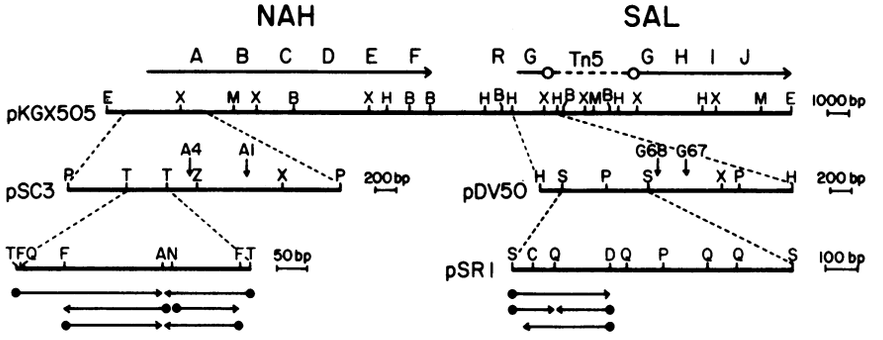

FIG. 1. Physical and genetic map of *nah* and *sal* operons and promoter subclones. Upper portion shows approximate location of the catabolic genes of the *nah* and *sal* operons (indicated by the capital letters A–F and G–J, respectively) and transcripts (→) in relationship to a physical map of pKGX505 (3, 9, 12). Below this are the physical maps of pSC3 (the *Hin*dIII site in the polylinker of pSC3 next to the left-hand *Pst* I site used in S1 nuclease mapping experiments is not shown) and pDV50 with the location of the most 5′ proximal Tn*5* insertions in *nahA* (A4, A1) and *nahG* (G68, G67) (3). At the bottom left is the physical map of the 390-bp *Bst*EII fragment of pSC3 containing the *nahA* promoter and sequencing strategy. At the bottom right is the physical map of pSR1 with sequencing strategy used for the 272-bp *Sal* I–*Dde* I fragment containing the *nahR* and *sal* transcription start sites. Restriction endonuclease site designations: E, *Eco*RI; X, *Xho* I; H, *Hin*dIII; B, *Bam*HI; P, *Pst* I; T, *Bst*EII; Q, *Taq* I; F, *Hin*fI; A, *Alu* I; N, *Nar* I; S, *Sal* I; D, *Dde* I; C, *Aha* III; Z, *Sph* I; M, *Sma* I.

ants of *E. coli* JM83 isolated on L agar containing ampicillin and X-Gal contained a plasmid with the 800-bp *Sal* I fragment of pDV50 inserted in pUC8, which was designated pSR1 (Fig. 1).

S1 Nuclease Mapping. RNA was prepared from *Pseudomonas putida* (NAH7) cells grown in L broth in the presence (induced) or absence (uninduced) of 0.05% sodium salicylate by a modification of the hot phenol extraction method (15). RNA (100 μg) was mixed with 5×10^5 cpm of DNA (*ca.* 0.1 μg) uniquely labeled at one 5′ end (see below). After ethanol precipitation, the pellet was redissolved in 30 μl of 80% formamide, 40 mM Pipes/KOH (pH 6.4), 0.4 M NaCl, and 1 mM EDTA, denatured at 85°C for 5 min, and hybridized overnight at 55°C. Reaction mixtures were combined with 0.3 ml of S1 nuclease buffer (0.28 M NaCl/50 mM sodium acetate, pH 4.6/5 mM $ZnSO_4$) containing 300 units of S1 nuclease per ml and 20 μg of denatured calf thymus DNA per ml and incubated at 37°C for 60 min, and the resultant products were analyzed on denaturing urea/polyacrylamide gels.

DNA Sequencing. Gel-purified DNA fragments were treated with bacterial alkaline phosphatase and were 5′ end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. After digestion with an appropriate restriction enzyme each uniquely end-labeled fragment was purified by electrophoresis on polyacrylamide gels. Fragments were sequenced by the chemical cleavage method of Maxam and Gilbert (16). A + G reactions were accomplished with formic acid (17). Gel electrophoresis was carried out in polyacrylamide gels in TBE buffer (89 mM Tris/90 mM boric acid/2 mM EDTA, pH 8.1); denaturing gels also contained 8 M urea. Sequencing strategy is shown in Fig. 1.

Sequence Analysis. Homologies between various DNA sequences were determined by a computer sequence analysis package on a PDP-11 computer (18). Genetic distances were calculated by utilizing Sankoff's algorithm (19).

Recombinant DNA Techniques. Basic techniques (e.g., plasmid preparation, gel electrophoresis, restriction endonuclease mapping, 5′ end-labeling, ligation, electroelution, etc.) are described in ref. 20.

RESULTS

Cloning of Promoter Fragments and S1 Nuclease Mapping of Transcripts. Polar effects of Tn*5* insertions in NAH7 on

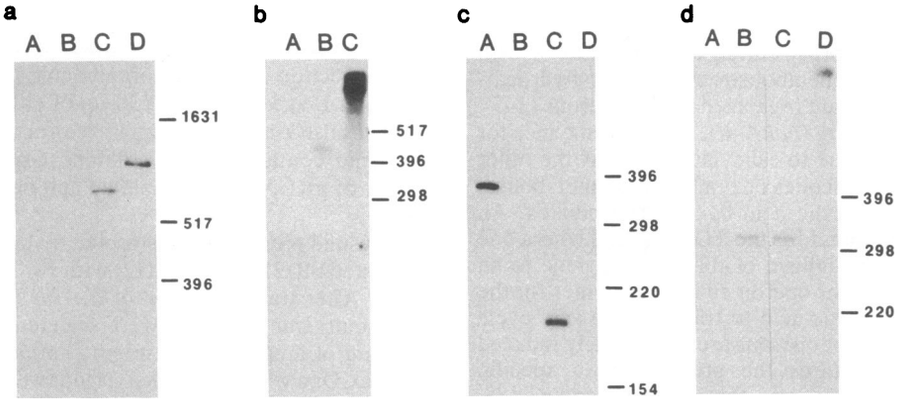

FIG. 2. S1 nuclease mapping of NAH7 transcripts from *P. putida*. RNA from salicylate-induced or noninduced *P. putida* (NAH7) cells was hybridized with DNA fragments labeled at a unique 5′ end, treated with S1 nuclease, electrophoresed on denaturing 8% polyacrylamide/urea gels, and autoradiographed. (*a*) S1 nuclease digestion products of the 780-bp *Aha* III–*Sal* I fragment of pSR1 5′ end-labeled at *Sal* I hybridized to *E. coli* tRNA (lane A), noninduced RNA (lane B), induced RNA (lane C), and untreated fragment (lane D). (*b*) S1 nuclease digestion products of the 1.2-kb *Hin*dIII–*Sph* I fragment of pSC3 5′ end-labeled at the *Sph* I site hybridized to noninduced RNA (lane A), induced RNA (lane B), and untreated fragment (lane C). (*c*) S1 nuclease digestion products of the 370-bp *Taq* I–*Bst*EII fragment of pSC3 5′ end-labeled at *Bst*EII hybridized to untreated fragment (lane A), noninduced RNA (lane B), induced RNA (lane C), and *E. coli* tRNA (lane D). (*d*) S1 nuclease digestion products of the plasmid pDV50 digested with *Hin*dIII 5′ end-labeled and hybridized with *E. coli* tRNA (lane A), induced RNA (lane B), noninduced RNA (lane C), and untreated fragment (lane D). The migration of molecular weight standards (pBR322 digested with *Hin*fI) is indicated at right.

expression of the catabolic operons suggested approximate locations for the *nah* and *sal* promoters (3). Therefore, the DNA fragments that included the most 5′ proximal Tn*5* insertion site and at least 1 kb of DNA upstream of it were cloned. Plasmid pSC3 contains a 2.7-kb *Pst* I fragment of pKGX530, which has 1.2 kb of DNA 5′ to the location of the nearest Tn*5* insertion in *nahA* (A4, Fig. 1), whereas plasmid pDV50 contains the 2.4-kb *Hin*dIII fragment of pKGX505, which contains part of the Tn*5* insertion in *nahG* (G67, Fig. 1). An 800-bp *Sal* I fragment 5′ to this Tn*5* insertion was subsequently cloned (pSR1).

RNA protection experiments were performed with plasmids pSC3 and pSR1 to precisely locate the 5′ ends of the *nah* and *sal* transcripts within 30 bp. Analysis of pSR1 for the *sal* transcription start (Fig. 2*a*) shows that a 620-bp RNA-protected DNA fragment was obtained after hybridization of salicylate-induced RNA to a 780-bp *Aha* III–*Sal* I fragment 5′ end-labeled at the *Sal* I site and subsequent digestion with S1 nuclease. The amount of 620-bp RNA-protected fragment was reduced by a factor of at least 20 when uninduced RNA from *P. putida* (NAH7) was used, because it was detected only after a much longer exposure (data not shown). This result is consistent with the inducible nature of *sal* transcription (9) and places the transcription site for the *sal* operon ≈50 bp downstream from the first *Taq* I site.

Similar analysis of pSC3 for the *nah* operon transcription start site utilizing a 1.2-kb *Hin*dIII–*Sph* I fragment of pSC3 5′ end-labeled at the *Sph* I site produced an RNA-protected fragment of 450 bp (Fig. 2*b*). Thus, the transcription start site for *nah* is near the middle of the 390-bp *Bst*EII fragment of pSC3. To map this start site more accurately, a 370-bp *Taq* I–*Bst*EII fragment 5′ end-labeled at *Bst*EII was used in S1 nuclease protection experiments (Fig. 2*c*). A 195-bp protected fragment was found after hybridization with salicylate-induced RNA from *P. putida* (NAH7). The amount of protected fragment was reduced by a factor of 20 when uninduced RNA was used, although the same size protected fragment was obtained (data not shown). This places the transcription start site *ca.* 60 bp upstream of the *Alu* I site of the cloned NAH7 fragment on pSC3 (Fig. 1).

The S1 nuclease protection assay was used to determine the direction and approximate start site of transcription of *nahR*, which maps near *nahG* (4, 9). Plasmid pDV50 was 5′ end-labeled, hybridized with RNA, and treated with S1 nuclease. An RNA-protected fragment of 320 bp was observed at the same intensity in reactions with either induced or uninduced RNA from *P. putida* NAH7 (Fig. 2*d*). This is consistent with constitutive transcription of *nahR* reported previously (9). This result places the start site of *nahR* transcription *ca.* 60 bp downstream of the *Aha* III site on the 800-bp *Sal* I fragment of pSR1. The *nahR* promoter is apparently located ≈50 bp from the *sal* promoter; however, its transcription proceeds in the opposite direction.

Sequence of Transcription Start Sites. The nucleotide sequence of the *nah* and *sal*/*nahR* promoter regions was determined. The 390-bp *Bst*EII fragment of pSC3 containing the transcription start of *nah* (Figs. 1 and 3*b*) and the 273-bp *Sal* I–*Dde* I fragment of pSR1 containing the transcription start sites of both *nahR* and the *sal* operon were sequenced (Figs. 1 and 3*a*). The A + T content of the middle 150 bp near the transcription start sites is about 65%, in contrast to the value of 33% found for NAH7 (21) or for a portion of the *nahG* structural gene (unpublished), strongly suggesting that the *nah* and *sal* promoters were located on these fragments.

To align the promoter sequences and determine homologous regions potentially involved in *nahR* regulation of the *nah* and *sal* operons, it was necessary to pinpoint the transcription start sites by comparison of S1 nuclease-digested RNA-protected 5′ end-labeled fragments to the Maxam–Gilbert chemical cleavage sequencing reactions of

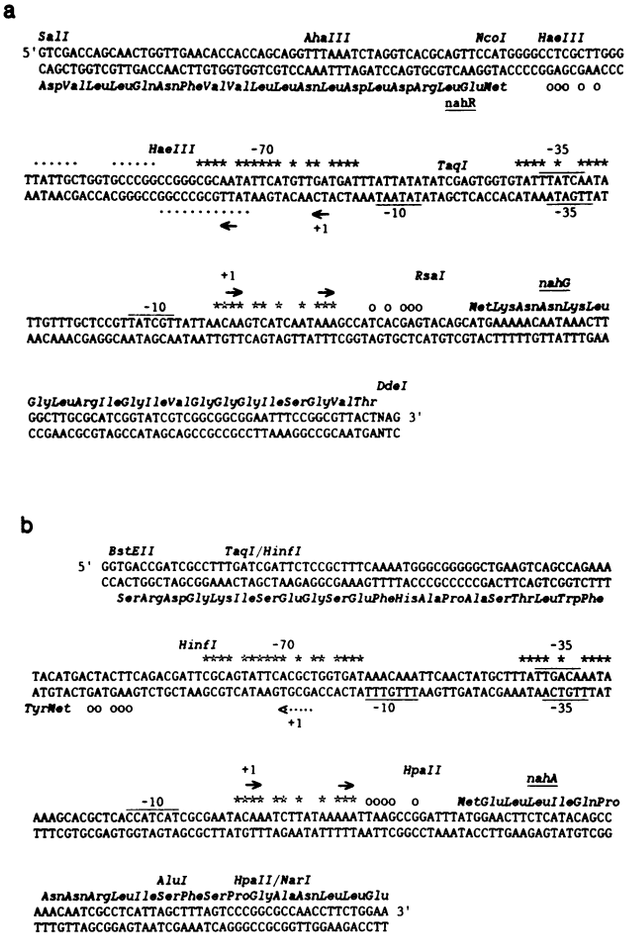

FIG. 3. Nucleotide sequence of the promoter regions of *nah*, *sal*, and *nahR*. (*a*) The nucleotide sequence of the 273-bp *Sal* I–*Dde* I fragment of pSR1. (*b*) The nucleotide sequence of the first 262 bp of the 390-bp *Bst*EII fragment of pSC3. The sequences are aligned by the putative translation initiation codons for *nahG* and *nahA*. The locations of the apparent transcription start sites are indicated by arrows; arrows with the designation +1 indicates the probable start of mRNA; the putative −35 and −10 consensus sequences are indicated by horizontal lines; open circles indicate bases with complementarity to *Pseudomonas aeruginosa* 16S RNA. Solid dots indicate the region of hyphenated dyad symmetry. The putative NH_2-terminal sequences of salicylate hydroxylase (*nahG*), naphthalene dioxygenase (*nahA*), and *nahR* gene product are shown. Bases marked with asterisks show regions of extensive homology between *nah* and *sal* promoter regions. The putative divergent transcription start site (···) and putative NH_2-terminal sequence of the gene product analogous in location to *nahR* are also shown.

the same fragments (Fig. 4 *a* and *b*). There appear to be possibly two transcription start sites for the *nah* and *sal* operons, because two protected fragments differing by 10 bp are evident. However, the regions following the first transcription start sites are unusually (A + T)-rich (75% for *sal* and 94% for *nah*), in contrast to the translated portions of the mRNAs, and perhaps form a weak RNA·DNA hybrid that is partially susceptible to S1 nuclease digestion.

Twelve base pairs upstream of the first *sal* transcription start site is the sequence TATGCT, of which 4 bp match the "−10" consensus sequence of *E. coli* promoters (22) (Fig. 3). Thirty-four base pairs upstream of the start site is the sequence TTATCA, of which 4 bp match the "−35" consensus sequence of *E. coli* promoters. The spacing between these sequences is 16 bp, which is also consistent with that found for *E. coli* promoters. Similarly, 14 bp upstream of the first transcription start site of *nah* is the sequence CATCAT,

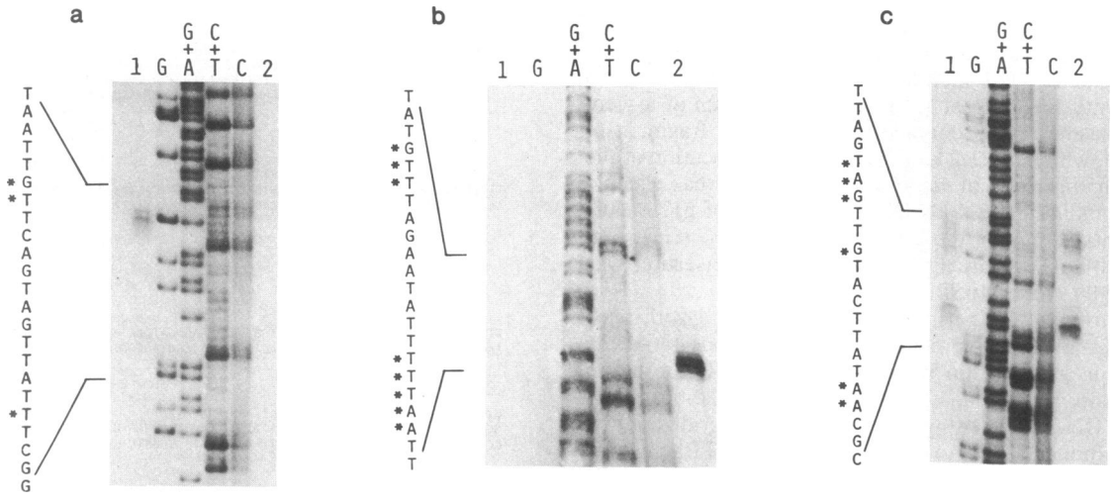

FIG. 4. Location of transcription start sites of *nah*, *sal*, and *nahR* by autoradiographic analysis of an 8% polyacrylamide/urea gel of S1 nuclease-treated hybridizations of salicylate-induced and noninduced RNA with 5' end-labeled *nah*, *sal*, and *nahR* promoter fragments. (*a*) S1 nuclease digestion products of induced RNA (lane 1) or noninduced RNA (lane 2) from *P. putida* (NAH7) hybridized with a 155-bp *Taq* I–*Dde* I fragment of pSR1 5' end-labeled at the *Dde* I site. The middle lanes (G, G + A, C + T, C) are the base-specific, chemical-cleavage sequencing reactions of the same fragment. The nucleotide sequences shown at the left of each gel have asterisks marking each apparent transcription start site. (*b*) S1 nuclease digestion products of noninduced RNA (lane 1) or induced RNA (lane 2) hybridized with a 360-bp *Taq* I–*Bst*EII fragment of pSC3 uniquely end-labeled at the *Bst*EII end. Middle lanes contain base-specific cleavage reactions. (*c*) S1 nuclease digestion products of hybridization of a 125-bp *Taq* I–*Sal* I fragment of pSR1 uniquely 5' end-labeled at the *Sal* I site and hybridized to induced RNA (lane 1) or noninduced RNA (lane 2) from *P. putida* (NAH7). Sequence reactions of the same fragment are located in the center of the gel.

with 4 of the 6 bp matching the *E. coli* consensus sequence, whereas 37 bp upstream of the transcription start site is the sequence TTGACA, which is an exact match of the *E. coli* −35 consensus sequence; the spacing of 17 bp between these two regions is consistent with *E. coli* promoter structure.

Approximately 30 bp downstream of the first transcription start site for each operon is an AUG translation initiation codon (Fig. 3). Eight base pairs upstream of the AUG is a 5-bp sequence complementary to the end of the 16S rRNA of *P. aeruginosa* (Shine–Dalgarno sequence) (23). Following the putative initiation codon for *nahA* is 150 bp of open reading frame (ORF) extending at least to the end of the sequenced fragment, which is ≈150 bp from the nearest Tn*5* insertion in *nahA* (3). Following the putative initiation codon for *nahG* is 60 bp of ORF extending at least to the end of the fragment, which is 500 bp from the nearest Tn*5* insertion in *nahG* (3).

Location of *nahR* Promoter. The precise transcription start site of the *nahR* gene was also identified by comparison of small RNA-protected fragments to the chemical-cleavage sequence ladder (Fig. 4*c*). As before, there appear to be two transcription start sites. Again, the region between the two apparent start sites is 80% A + T and may produce anomalously shorter fragments. The first transcription start site is followed by a putative AUG translation initiation site that is preceded by a 6-bp sequence complementary to the 16S rRNA of *P. aeruginosa*. Twelve base pairs upstream of the first transcription start site is the sequence TATAAT, which perfectly matches the −10 consensus sequence of *E. coli* promoters. Thirty-four base pairs upstream is the sequence TTGATA, of which five bases match the −35 consensus sequence of *E. coli* promoters; this sequence also overlaps the proposed −35 sequence for the *sal* operon. The putative AUG initiation codon is followed by 56 bp of ORF extending at least to the end of the sequenced fragment, which is ≈150 bp from the first Tn*5* insertion in *nahR* (4). In addition, this 150-bp region has been shown to be required for function of *nahR* (9).

The nucleotide sequence following the first *nahR* transcription site contains a long region of hyphenated dyad symmetry (Fig. 3*a*; +12 to +41 of *nahR*). A large stem–loop structure could exist in this region of the *nahR* transcript since a very stable structure (−35 kcal/mol; 1 cal = 4.184 J) can be hypothetically drawn for this sequence (24). The significance or function of the hypothetical structure is not known, although it could be involved in modulation of *nahR* expression.

Sequence Homology Between *nah* and *sal*. Analysis of the sequences of the promoter regions of *nah* and *sal* after alignment by the putative AUG translation initiation codons of their respective mRNAs showed three areas of extensive homology (Fig. 3): (*i*) in the region between −60 and −80 before the first transcription start, 17 of 21 bases were homologous; (*ii*) in the −26 to −37 region for *sal* and −28 to −39 region for *nah*, 9 of 12 bases were homologous [within these four regions the common core sequence TATTNAY (where Y = unknown pyrimidine) was found]; (*iii*) in the 16-bp region where the transcription start sites were located, 11 of 16 bp preceding the Shine–Dalgarno sequence were homologous.

Comparison of the 90-bp sequences preceding the first transcription start sites of *nah* and *sal* with the analogous sequences of the *xylABC* and *xylDEFG* operons of TOL plasmid (10, 11) showed no significant homologous regions (i.e., >4 of 7 bases matching). Comparison with the promoter sequence of the positively controlled *araBAD* operon (25) or *malEFG* operon (26) of *E. coli* likewise showed little significant homology. Comparison of the *nah* promoter with the *gal* P_1 promoter of *E. coli* (27) did show significant homology in that 11 of the first 14 bases before the transcription start site are homologous.

DISCUSSION

The locations of the transcription start sites of the *nah* and *sal* operons as well as their common regulatory gene *nahR* were determined by S1 nuclease mapping and comparison to the nucleotide sequences surrounding the transcription start sites. The results may suggest that there are two distinct sites (separated by *ca.* 10 bp) where transcription is initiated for either the *nah* or *sal* operon. However, due to the contrast between the extremely high A + T content of the region between the two apparent transcription start sites and the high G + C content of the remainder of the mRNA it is possible that this is an artifact of the S1 nuclease mapping

technique. The appropriate location of *E. coli*-type −35 and −10 sequences before the first transcription start sites is consistent with the hypothesis that the second apparent transcription start sites are RNA·DNA hybridization artifacts. Although the apparent presence of two transcription start sites is similar to that reported by Mermod *et al.* for the analogous *xylDEFG* promoter of the TOL plasmid (28), the region between these two transcription start sites is also (A + T)-rich. In contrast, Inouye *et al.* (11) have detected only a single transcription start site for the same *xylDEFG* promoter by utilizing reverse transcriptase analysis.

After alignment of the *nah* and *sal* promoter sequences, two 5′ flanking regions of extensive homology were found: a 12-bp sequence at −35 (the putative RNA polymerase binding site) and a 21-bp sequence at −70. It is tempting to suggest that the homologous sequences around −70 are the binding sites for the transcriptional activator product of the *nahR* gene since the −60 to −80 region has been found to be the site of action of other positive regulatory gene products: *araC* (27) and *malT* (26). In fact, recent analysis of plasmids with various upstream portions of the *sal* promoter fused to the *E. coli* galactokinase gene has shown that the region between −83 and −45 is absolutely required for *nahR*-mediated *trans*-activation of the expression of the *sal* operon by salicylate (unpublished data). DNase "footprinting" experiments with the *nahR* gene product or *in vitro* mutagenesis of this nucleotide sequence will be necessary to confirm and further delineate the site of action of the *nahR* gene product.

Comparison of the *nah* or *sal* promoter region sequences to the analogous sequences of the *xylDEFG* and *xylABC* (10, 11) showed very little homology or similarities. The genetic distances between the *xylABC* promoter region and the *nah* or *sal* promoter regions (i.e., 115 bp preceding AUG start codons) are 77 and 78, respectively, whereas the genetic distance between two random 115-bp sequences is 80. No homologies between the consensus sequences of *P. putida* promoters proposed by Mermod *et al.* (28) or Inouye *et al.* (11) were observed. Although TOL and NAH7 catabolic operons are organized and regulated by analogous positive regulatory genes, the promoter sequences are substantially different. The regulatory systems for each degradative plasmid system appear to have evolved separately. On the other hand, the high level of homology between the promoter regions of *nah* and *sal* suggest a common ancestor or, perhaps, that the *nah* promoter evolved from the *sal* promoter sequence. In support of this hypothesis, the genetic distance between the two 115-bp sequences preceding the translation initiation codons of *nahA* and *nahG* is 65, whereas the genetic distance between two random 115-bp sequences is 80 (19).

The finding of what appear to be *E. coli*-like promoter sequences for *nah, sal,* and *nahR* and sequences with complementarity to *E. coli* 16S rRNA probably explains the ability of the NAH7 genes to be expressed and properly regulated in *E. coli* (9). It also suggests that these genes could have a broad host range and may also be capable of regulated expression in other Gram-negative bacteria. This is in contrast to the findings of Inouye *et al.* (10, 11) for the TOL plasmid operons *xylABC* and *xylDEFG*. Although the TOL genes are expressed and regulated in *E. coli*, their promoter sequences are different from those of *E. coli*. However, *P. putida* and *E. coli* RNA polymerases apparently recognize similar sites on the DNA (29); thus, it is important to consider the three-dimensional structure of the promoter regions, not just their DNA sequence.

The arrangement of the *sal* and *nahR* divergent promoters is unusual since they apparently share a −35 region. This sharing may be involved in regulation or may indicate that the *nahR/sal* system evolved before the *nah* + *sal* naphthalene degradation system. It is important to note that a divergent, "−35 overlapping" promoter analogous to that found for *nahR* may exist at the *nah* promoter (Fig. 3*b*) since appropriately spaced sequence elements (i.e., −35 and −10 sequences, ribosome binding site, and AUG initiation codon, ORF) are found there.

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