The $\sigma^{54}$-factor controls expression of a variety of genes in response to environmental cues. Much previous work has implicated the nucleotide alarmone ppGpp and its co-factor DksA in control of $\sigma^{54}$-dependent transcription in the gut commensal Escherichia coli, which has evolved to live under very different environmental conditions than Pseudomonas putida. Here we compared ppGpp/DksA mediated control of $\sigma^{54}$-dependent transcription in these two organisms. Our in vivo experiments employed P. putida mutants and manipulations of factors implicated in ppGpp/DksA mediated control of $\sigma^{54}$-dependent transcription in combination with a series of $\sigma^{54}$-promoters with graded affinities for $\sigma^{54}$-RNA polymerase. For in vitro analysis we used a P. putida-based reconstituted $\sigma^{54}$-transcription assay system in conjunction with DNA-binding plasmon resonance analysis of native and heterologous $\sigma^{54}$-RNA polymerase. In comparison with E. coli, ppGpp/DksA responsive $\sigma^{54}$-transcription in the environmentally adaptable P. putida was found to be more robust under low energy conditions that occur upon nutrient depletion. The mechanism behind this difference can be traced to reduced promoter discrimination of low affinity $\sigma^{54}$-promoters that is conferred by the strong DNA binding properties of the P. putida $\sigma^{54}$-RNA polymerase holoenzyme.

To ensure promoter specificity, the bacterial multisubunit catalytic core RNA polymerase (RNAP)$^5$ ($\alpha_{54}\beta\beta'\omega$) is guided to the distinct promoter classes within the genome by a dissociable $\sigma$-factor. In addition to the household $\sigma^{70}$-factor, most bacteria encode a variable number of alternative $\sigma$-factors that fall into two families based on primary amino acid sequences and regulatory properties. The largest of these is the $\sigma^{70}$-family, which encompasses most alternative $\sigma$-factors within four subgroups that all form RNAP holoenzymes that can spontaneously initiate transcription (1). Orthologs of the structurally distinct $\sigma^{54}$-subunit are the only members of the second family. $\sigma^{54}$ imposes kinetic constraints that lock the $\sigma^{54}$-RNA holoenzyme in a non-productive closed complex. Thus, $\sigma^{54}$-dependent transcription always requires activators, called bacterial enhancer-binding proteins or bEBPs, which use ATP catalysis to remodel $\sigma^{54}$-RNAP/DNA interactions to stimulate DNA melting and formation of the transcription competent open complex (reviewed in Ref. 2).

Many bacteria from different phylogenetic groups use $\sigma^{54}$-dependent transcription to control numerous environmental-responsive processes ranging from expression of chemotaxis transducers and assembly of motility organs, through alginate biosynthesis, to nitrogen assimilation and the utilization of different carbon sources (Refs. 2–4 and references therein). The metabolically and environmentally versatile Pseudomonas putida species use $\sigma^{54}$-dependent transcription extensively (5). This group of organisms, in combination with resident catabolic plasmids, is notably associated with its ability to use aromatic compounds as growth substrates. In many cases, the expression of these pathways are ultimately dependent on $\sigma^{54}$-RNAP and are subject to global regulatory input. This results in suppression of transcription of the specialized catabolic genes when energetically more favorable carbon sources are present (reviewed in Refs. 6 and 7). One such example is the dimethylphenol dmp-system of the plasmid pVI150, in which transcription of the dmp-operon for production of the catabolic enzymes is driven by the $\sigma^{54}$-Po promoter. Transcription from $\sigma^{54}$-Po is controlled in response to the presence of phenolic dmp-pathway substrates through the action of the divergently transcribed dmpR gene product, which binds the aromatic compounds to achieve its active transcriptional-promoting form (8–10).

For the DmpR-regulated $\sigma^{54}$-Po promoter, the bacterial alarmone ppGpp acts together with DksA to provide a link between $\sigma^{54}$-dependent transcription and the metabolic status of the cell. This dominant global regulation results in silencing of Po activity until nutrient depletion at the exponential to stationary growth phase transition (11–14). In Escherichia coli, the levels of ppGpp vary enormously and are rapidly elevated in response to both nutritional limitation and physicochemical stress to redirect transcriptional capacity toward genes required to counteract adverse conditions (reviewed in Ref. 15). Both ppGpp and DksA directly target RNAP so that the relatively constant levels of DksA sensitize RNAP to the cellular levels of ppGpp to directly inhibit or stimulate transcription from susceptible $\sigma^{54}$-dependent promoters of E. coli (16, 17). Lack of ppGpp and/or DksA severely reduces $\sigma^{54}$-dependent Po activ-

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9 The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1 and Figs. S1 and S2.

10 Both authors contributed equally to this work.

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13 The abbreviations used are: RNAP, RNA polymerase; bEBP, bacterial enhancer-binding protein; ppGpp, guanosine bispyrophosphate; IPTG, isopropyl β-d-thiogalactopyranoside; IHF, integration host factor; UAS, upstream activation site; Km, kanamycin; Tel, tellurite.


### **TABLE 1**

**Bacterial strains**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Relevant properties</th>
<th>Ref./Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL21(DE3) pLysS</td>
<td>IPTG inducible T7 polymerase expression host</td>
<td>54</td>
</tr>
<tr>
<td>DH5</td>
<td>Prototrophic, Res</td>
<td>55</td>
</tr>
<tr>
<td>MG1655</td>
<td>K12, prototrophic</td>
<td>56</td>
</tr>
<tr>
<td>MG1655-Δrdsl::Km</td>
<td>Km(^{\text{R}}), gene replacement of rdsl of MG1655</td>
<td>S. Busby</td>
</tr>
<tr>
<td>MG1655-Δp_scAB</td>
<td>Km(^{\text{R}}), monopsony P(_{\text{scAB}})-luxAB transcriptional reporter strain</td>
<td>A. Åberg</td>
</tr>
<tr>
<td>RK201</td>
<td>Km(^{\text{R}}), MG1655-ΔdksA::Km, DksA null</td>
<td>57</td>
</tr>
<tr>
<td>S17Δpir</td>
<td>Tp(^{\text{R}}), Sm(^{\text{R}}), Res(^{\text{R}}), R48-mob(^{\text{R}}), Apir lysogen</td>
<td>58</td>
</tr>
<tr>
<td>P. putida strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KT2440</td>
<td>Prototrophic</td>
<td>59</td>
</tr>
<tr>
<td>KT2440-rpoN::Tn5</td>
<td>Km(^{\text{R}}), σ(^{54})-null mutant of KT2440</td>
<td>60</td>
</tr>
<tr>
<td>KT2440-Δp_scAB</td>
<td>T(_E), KT2440-Δp_scAB tagged with miniTn5-Tel</td>
<td>30</td>
</tr>
<tr>
<td>KT2440-Δp_scAB-luxAB</td>
<td>T(<em>E), Km(^{\text{R}}), monocopy P(</em>{\text{scAB}})-luxAB transcriptional reporter of KT2440-Tel</td>
<td>This study</td>
</tr>
<tr>
<td>KT2440-ΔdmpR-Tel</td>
<td>Tel(^{\text{R}}), dmpR transcribed from its native promoter on miniTn5-Tel</td>
<td>30</td>
</tr>
<tr>
<td>KT2440-ΔdmpR-Δp_scAB::Hfr</td>
<td>Tel(^{\text{R}}), T(_C), KT2440-ΔdmpR-Tel with lux(^{\text{R}})/p_scAB-luxAB on miniTn5-Tc</td>
<td>30</td>
</tr>
<tr>
<td>KT2440-ΔdmpR-Δp_scAB::Hfr::Km</td>
<td>Tel(^{\text{R}}), Km(^{\text{R}}), gene replacement of P(_{\text{scAB}}) in KT2440-ΔdmpR-Tel</td>
<td>This study</td>
</tr>
<tr>
<td>KT2440::xylR-Tel</td>
<td>Tel(^{\text{R}}), xylR transcribed from its native promoter on miniTn5-Tel</td>
<td>30</td>
</tr>
<tr>
<td>PP1::ΔdmpR</td>
<td>Tel(^{\text{R}}), Km(^{\text{R}}), Gm(^{\text{R}}), ppGpp(^{\text{R}}) KT2440-ΔdmpR-Tel</td>
<td>30</td>
</tr>
<tr>
<td>PP1::xylR</td>
<td>Tel(^{\text{R}}), Km(^{\text{R}}), Gm(^{\text{R}}), ppGpp(^{\text{R}}) KT2440::xylR-Tel</td>
<td>30</td>
</tr>
<tr>
<td>PP2::ΔdmpR</td>
<td>Tel(^{\text{R}}), T(_C), KT2440ΔdmpR-Tel, ΔdksA::Tc</td>
<td>14</td>
</tr>
</tbody>
</table>

* Resistance abbreviations are: Cm, chloramphenicol; Km, kanamycin; Gm, gentamycin; Tc, tetracycline; Tel, tellurite; Sm, streptomycin; Tp, trimethoprim.

ity in both *E. coli* and *P. putida* without altering the constant levels of σ\(^{54}\) in the cell (11–14). Artificial synthesis of ppGpp during exponential growth on rich media, on the other hand, results in transcription from Po under conditions where it is normally silent (11–13). However, whereas ppGpp and/or DksA-deficiency in *E. coli* severely limit transcription from Po and a range of other σ\(^{54}\)-dependent promoters in *E. coli*, they do not have any apparent effects in an *E. coli* reconstituted in vitro σ\(^{54}\)-dependent transcription system (13). Together with the demonstration that σ\(^{54}\)-RNAP levels are limiting in the cell (12), and the properties of RNAP mutations that bypass the need for ppGpp for efficient σ\(^{54}\)-dependent transcription, these findings have led to a model in which ppGpp synthesis indirectly results in elevated levels of σ\(^{54}\)-RNAP through competition between σ\(^{54}\) and other σ-factors for varying levels of core RNAP that are available for holoenzyme formation (12, 13, 18).

DksA and ppGpp have also been found to play other roles in the DmpR/α\(^{54}\)-Po regulatory circuit that would also contribute to the abrupt high level transcription observed from Po at the exponential to stationary phase growth transition (14). First, ppGpp and DksA directly stimulate transcription by σ\(^{70}\)-RNAP from the Pr promoter of the *dmpR* gene to elevate DmpR levels (14). Second, ppGpp and DksA might also elevate the levels of IHF (integration host factor) that facilitates productive interaction between Po-bound σ\(^{54}\)-RNAP and DmpR bound to its distally located binding sites (upstream activation sites, UASs) (19). Third, DmpR also stimulates its own production through a mechanism in which σ\(^{54}\)-RNAP activity at the σ\(^{54}\)-Po promoter stimulates σ\(^{54}\)-Pr output. This regulation thus generates a feed forward loop that is dependent on not only the direct effects of ppGpp and DksA on Pr activity, but also their effects on IHF and σ\(^{54}\)-RNAP levels required for Po activity (14).

Much of the analysis of the mechanisms of ppGpp/DksA-mediated control of σ\(^{54}\)-transcription have been identified using mutants of *E. coli*, which has a very different lifestyle than the environmentally and nutritionally adaptable soil Pseudomonas. In addition to bouts of feast and famine, soil bacteria are frequently exposed to extremes in physicochemical parameters (temperature, water content etc). When faced with aromatic compounds, bacteria are confronted with chemicals that, can potentially be catabolized as a carbon and energy source for growth, but are also toxic stress agents (Ref. 20 and references therein). Transcription from σ\(^{54}\)-promoters, as with any other type of promoter, functions within the context of the evolutionary selected regulatory network of the host bacteria. In this work we sought to test if regulatory mechanisms that couple σ\(^{54}\)-dependent transcription to cellular physiology in *E. coli* also operate in *P. putida*. In particular, we were interested to determine whether the properties of the σ\(^{54}\)-RNAPs from *E. coli* and *P. putida* underlie differences in ppGpp/DksA control of σ\(^{54}\)-promoter activity that we observed in vivo in these two organisms.

### EXPERIMENTAL PROCEDURES

**General Procedures**—*E. coli* and *P. putida* strains (Table 1) were grown in Luria-Bertani medium (LB), (21) supplemented with the following antibiotics as appropriate for the strain and resident plasmid selection: carbenicillin (Cb, 100 µg ml\(^{-1}\)) for *E. coli* and 2 mg ml\(^{-1}\) for *P. putida); kanamycin (Km, 50 µg ml\(^{-1}\) for both *E. coli* and *P. putida*), tellurite (Tel, 40 µg ml\(^{-1}\) for *P. putida*), tetracycline (Tc, 5 µg ml\(^{-1}\) for *E. coli* and 50 µg ml\(^{-1}\) for *P. putida*), and trimethoprim (Tp, 100 µg ml\(^{-1}\) for *E. coli*). M9 minimal media plates (21) supplemented with 10 mM glucose and 100 µg ml\(^{-1}\) thiamine were used for prototrophy tests. *E. coli* DH5 was used for construction and maintenance of all but R6K-based suicide plasmids for which the replication permissive S17Δpir host was used.

**Plasmids and DNA Manipulations**—Plasmids were constructed by using standard DNA techniques. The fidelity of all DNA sequences of PCR-generated fragments and linkers was confirmed. Luciferase (luxAB) transcriptional reporters and plasmids used in *in vitro* transcription assays are listed in Table 2, whereas primers used are listed in supplemental Table S1.

**luxAB Transcriptional Reporter Plasmids**—Key DNA features of the different luciferase transcriptional reporters used in this study are shown in Fig. 1. Plasmids were constructed by a common procedure of assembly on pBluescript SK+ (Stratagene) followed by subcloning into an RSF1010-based plasmid.
to generate the broad host range (16 to 20 copies per cell) transcriptional reporters (13). Construction of pBluescript-based plasmids pVI769 and pVI733 has previously been described (13). Plasmid pVI769 carries the *dmpR*-Po promoter region with unique NdeI and BamHI sites located −39 relative to the transcriptional start of Po for insertion of *α*-44 promoter regions upstream of the promoterless luxAB genes. Plasmid pVI733 carries the same DNA as pVI769 but with a non-native XhoI site engineered at −122 to −117 relative to the transcriptional start of Po. The XhoI to NdeI Po promoter upstream region of pVI733 was replaced by the equivalent region of Po using a custom designed linker, resulting in pVI1778. In the next step, linkers with the desired *α*-44 promoter sequences were introduced into pVI1778, as previously described (13), to give pVI1779 (xh-Po/Po-luxAB), pVI1780 (xh-Po/U-luxAB), pVI1781 (xh-Po/PpspA-luxAB), and pVI1782 (xh-Po/GlnA-luxAB). The promoter-luxAB fusions of pVI1779 to pVI1782 were then excised as XhoI to BamHI fragments using primers dksAup-f and dksAup-r and inserted between these sites of pET28a (Novagen), to give pVI1905.

**DksA Expression Plasmid**—The expression plasmid pVI1906 encoding *P. putida* His-DksA was then amplified as a KpnI to SacI fragment using primers dksApro-f and dksApro-r and inserted between these sites of pKm705L to generate pH9016 for subsequent single site recombination into the chromosome of *P. putida* as previously described (22).

**pfrA Gene Inactivation Plasmid**—To generate a gene replacement mutant of the *pfrA* gene of *P. putida* (TIGR PP0191), an XhoI to BamHI fragment spanning the 5′- and 3′-ends of the *pfrA* gene with a unique BglII site replacing codons 17 to 156 was generated by overlapping PCR using primers pfrAup-f, pfrAup-r, pfrAdo-f, and pfrAdo-r. The resulting XhoI to BamHI fragment was cloned between these sites of pBluescript SK+ (Strategene) to generate pVI910. A BamHI fragment carrying the Km gene of p34S-Km3 (23) was then cloned into the BglII site of pVI910, with the antibiotic resistance gene transcribing in the same direction as the *pfrA* gene, generating pVI910. Finally, the resulting Δ*pfrA:*Km gene replacement was cloned as an XhoI to BamHI fragment between the XhoI and BglII sites of the R6K suicide plasmid pDM4-Tc (22) resulting in pVI911.

**Strain Generation**—Introduction of the *lacIqR*::*lac-ihfAB mini-Tc transposon carried on the suicide plasmid pUTtetPF (24) into the chromosome of *P. putida* KT2440::*dmpR*-Tel was by conjugation from the *E. coli* donor host S17Δ*pfr.* Tellurite and tetracycline were used as counter selection for the donor and recipient parental strains, respectively. To generate the Δ*pfrA:*Km kanamycin gene replacement mutant of *P. putida* KT2440::*dmpR*-Tel, pVI911 was introduced by conjugation from S17Δ*pfr*, with tellurite and kanamycin used as counter selection for the donor and recipient parental strains. Double site recombinants were identified by screening for loss of the plasmid-encoded Tc resistance marker, and verified by diagnostic PCR using primers specific for the intact *pfrA* gene and the Δ*pfrA:*Km kanamycin gene replacement.

**Luciferase Reporter Gene Assays**—Luciferase assays of the *luxAB* gene product were performed on cultures grown and assayed at 30°C. To ensure balanced growth, overnight cultures were diluted and grown into exponential phase prior to a second dilution to *A*₅₆₂ of 0.05 to 0.08 and initiation of the experiment by addition of the DmpR effector 2-methylphenol or the XylR effector 3-methylbenzylalcohol to a final concentration of 2 mM for *P. putida* strains and 0.5 mM for *E. coli* strains. When used, IPTG (isopropyl-β-D-thiogalactopyranoside) was present throughout growth from overnight cultures. Light emission from 100 μl of whole cells using 1:2000 dilution of decanal was measured using a PhL Luminometer (Aureon Biosystems). Data points are the average of duplicate determinations from each of two or more independent experiments.

**Immunoblot Analyses**—Crude extracts of soluble cytosolic proteins, SDS-PAGE separation, and electrophoretic transfer to membrane for immunodetection of proteins were as described previously (25). Rabbit polyclonal antiserum against *E. coli* DksA (gift from D. Downs, Wisconsin) was pre-cleared against total cell lysate of an *E. coli* DksA null mutant (RK201) prior to use. Other polyclonal antiserum used were directed against *P. putida* IHF (26), the amino-terminal 232 residues of DmpR (13) and against *P. putida* RpoN (14). Antibody-decorated bands were revealed using Amersham Biosciences polyvinylidene difluoride membrane and ECL+Plus reagents as directed by the manufacturer.

**ppGpp and Purified Proteins for in Vitro Assays**—The nucleotide ppGpp was synthesized from ribosome-associated RelA as previously described (27). *E. coli* core RNAp and *α* were as...
described in Ref. 13. *P. putida* IHF was a gift from F. Bartels. Native *P. putida* KT2440 core RNAP was purified as described in Ref. 28, whereas *P. putida* σ54 was purified after overexpression as previously described (14). *P. putida* His-DksA was expressed in BL21(DE3) pLysS harboring the *P. putida*-dksA plasmid pV1905. Cells were grown at 30 °C to A600 of 0.7 and expression induced by culturing in the presence of 0.5 mM IPTG for 3 h prior to harvesting the cells and purification of His-DksA by nickel affinity chromatography essentially as described previously for DmpR-His (9).

**In Vitro Transcription Assays—**Transcription assays employed supercoiled plasmids pV1736 to pV1741, which carry the −578 to +2 region of the σ54- promoters on pTE103 (29). Reactions were carried out at 30 °C in 20 μl of T-buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 1 mM dithiothreitol, 0.1 mM EDTA, and 0.275 mg/ml bovine serum albumin) as described in Ref. 13, except that *P. putida* transcriptional components were used. In brief, core RNA polymerase was mixed with the indicated molar excess of σ54 and incubated for 5 min to allow holoenzyme formation. When present, ppGpp (200 μM) and DksA (2 μM) were added and incubation continued for a further 5 min. Open complex formation was initiated by the addition of 0.5 mM supercoiled plasmid DNA, IHF (10 μM), DmpR-His (50 μM), the aromatic effector 2-methylnaphthalenol (0.5 mM) and ATP (4 mM) that are required for DmpR activity. Transcription was initiated by adding 2.5 μl of a mixture of ATP (final concentration, 0.4 mM), GTP and CTP (final concentration, 0.2 mM each), UTP (final concentration, 0.08 mM), and [α-32P]UTP (5 μCi at >3,000 Ci/mmol, Amersham Biosciences). In multiple round assays, heparin (0.125 mg/ml final concentration) was added after 5 min to prevent re-initiation and incubation continued for a further 5 min. In single round assays, heparin was added simultaneously with NTPs and reactions incubated for 10 min. After termination of the reactions, transcripts were analyzed on a 4% acrylamide gel containing 7 M urea, followed by quantification using phosphorimaging.

**Plasmon Resonance Assays—**Plasmon resonance experiments were performed using a Biacore 3000 system (Uppsala, Sweden). Biotin-labeled double-stranded DNA fragments (121 bp) encompassing the PnifH, Po, and PglnA σ54-promoters were coupled to streptavidin immobilized on a CM4 chip by amine coupling. The DNA fragments were generated from Po/Px-luxAB fusions by PCR amplification using primer 2200 (homologous to the Po upstream region) and the biotin-labeled primer 2201 (complementary to the luxAB genes). The experiments were run using buffer B (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 1 mM dithiothreitol, 0.1 mM EDTA, 0.054% P-Surfactant (Biacore)) at 25 °C. σ54-RNA polymerases were formed by preincubation of core RNA polymerase and an 8-fold excess of σ54 for 5 min in buffer B, then injected at a flow rate of 20 μl/min.

**RESULTS AND DISCUSSION**

**Growth Profiles of ppGpp- or DksA-deficient *P. putida*—**We have previously found that whereas the behavior of the σ54-Po promoter in *E. coli* generally reflects that observed in *P. putida*, the ppGpp-dependent profiles in the two strains differ (30).

Likewise, the growth properties of ppGpp-deficient *P. putida* differ from those of its *E. coli* counterpart. Consistent with its nutritional adaptability, ppGpp-deficient *P. putida* remains prototrophic, whereas ppGpp-deficient *E. coli* is polyauxotrophic (30). That this is a genuine difference between the two organisms, rather than due to a prototrophy-restoring mutation in the β or β’ (RpoBC) subunits of the core RNAP (31), was ensured both by the strategy used to generate the *P. putida* mutants and by confirming the wild-type sequence of the rpoBC genes in three independent isolates (30). This difference between *E. coli* and *P. putida* prompted us to also test DksA-deficient derivatives of *P. putida*. Like ppGpp-deficient *P. putida*, 10 independent derivatives of DksA-deficient *P. putida* were found to be prototrophic, which again contrasts the polyauxotrophic phenotype of DksA-deficient *E. coli* (32).

**Genetic Organization of Reporter Systems to Monitor σ54-transcription in *P. putida*—**Artificial manipulation of ppGpp levels or natural elevation of ppGpp in response to nutrient depletion at the exponential-to-stationary growth phase transition result in efficient transcription from the σ54-Po promoter in both *E. coli* and *P. putida* (11, 33). All that is needed to reproduce this metabolic coupling is the Po regulatory region and DmpR, either encoded in its native divergent configuration with respect to the Po promoter, or encoded on a separate replicon (30). To probe ppGpp- and DksA-mediated regulation of σ54-transcription in *P. putida* KT2440, we used derivatives that express the substrate-responsive βE2s DmpR or XylR from their native cistrons introduced into the host chromosome via mini-transposons. These strains were used in conjunction with a series of luciferase transcriptional reporter plasmids that were constructed within the framework of the σ54-Po promoter to maintain critical promoter architecture and relative spacing of the DNA binding sites of the proteins that control σ54-dependant transcription (13).

As depicted in Fig. 1, these promoters, designated by the Po/Px series, have the upstream region of the Po promoter (Po/−122 to −40) combined with different σ54-RNAP binding motifs in the −39 to +2 (/Px) regions. The −39 to +2 regions are from well studied σ54-promoters and include the *P. putida*-derived XylR-regulated Pu promoter of tolulene/xylene catabolism, the *Klebsiella pneumoniae*-derived nifH promoter and its mutant derivative nifh409, in which substitution of the −17 to −15 CCC by TTT increases affinity and transcriptional output, and the strong *E. coli*-derived pspA and glnA promoters (13). The relative affinities of *E. coli* σ54-RNAP for these six Po/Px promoters have previously been defined (13). The six −39 to +2 promoter sequences were further combined with either a mutated Po upstream region in which the IHF binding site consensus had been disrupted but that maintains the same base composition of Po to result in the xh-Po-(IHF)/Px series (xh indicates the presence of a non-native Xhol cloning site), or with the Pu promoter upstream region to result in the xh-Pu/Px series of transcriptional reporters.

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Both ppGpp and DksA Are Required for Robust Stationary Phase 54-promoter output from the luciferase 54-Po/Po promoter region with open arrowheads indicating the direction of transcription of coding regions. Promoter designations, Px/Px, indicate the origin and status of the upstream IHF binding site containing region (−122 to −40) downstream (−39 to +2) 54-RNAP binding region, with Xhol denoting the presence of a non-native Xhol site at −122 to −137. Coordinates are relative to the +1 transcriptional start of Po. Locations of the DNA binding sites for DmpR (inverted shaded arrows, UAS1, UAS2), the IHF recognition sequence (shaded box), and the −24, −12 Po promoter motif (open boxes) are indicated. Restriction sites used in DNA manipulations are indicated, with non-native sites given in brackets. The upper part of the figure gives the nucleotide sequences of the upstream region that vary in the different reporters compared with those of Po and Pu promoter regions. The IHF binding sites of Po (19) and Pu (52) are shown in bold and aligned with the consensus sequence that includes the core 5′-WATCAR—TTR-3′ motif (where W is A or T and R is A or G) separated from a less conserved A/T-rich tract of 4 to 6 bp (lowercase letters) (53). The residues shuffled to disrupt the core IHF consensus in the xh-Po(-IHF)/Px promoter series are compared with those of Po and Pu promoter regions. The IHF binding sites of Po (19) and Pu (52) are shown in bold and aligned with the consensus sequence that includes the core 5′-WATCAR—TTR-3′ motif (where W is A or T and R is A or G) separated from a less conserved A/T-rich tract of 4 to 6 bp (lowercase letters). The residues shuffled to disrupt the core IHF consensus in the xh-Po(-IHF)/Px promoter series are compared with those of Po and Pu promoter regions.

Both ppGpp and DksA are required for efficient 54-dependent transcription from this promoter (Fig. 2A). Consistent with our recent findings (14), the levels of DmpR vary across the growth curve in P. putida, reaching their highest levels in the post-exponential phase of growth (Fig. 2B and Ref. 14). Again, similar to the case in E. coli, the absence of either ppGpp or DksA did not influence the levels of 54-Po, whereas ppGpp deficiency (but not DksA deficiency) results in an ∼2-fold decrease in DmpR levels in both organisms (Fig. 2C and Ref. 13).

P. putida contains a homologue of both the E. coli RelA ppGpp synthetase I and the bifunctional SpoT ppGpp-hydrolase/synthetase II (30). It has previously been experimentally demonstrated that ppGpp levels are dramatically increased at the exponential to stationary phase transition in LB cultured P. putida (11). In E. coli, the levels of the DksA protein are relatively constant across the growth curve and under different growth conditions (16, 32, 34), whereas in P. aeruginosa, they have been found to vary (35). Therefore, it was of interest to compare transcriptional profiles and DksA protein levels in P. putida and E. coli. To this end we monitored transcription across the growth curve with derivatives of each species that each carried a transcriptional fusion of the luxAB reporter genes to the respective promoter of dksA on their chromosomes. As shown in Fig. 2D, transcription from the P. putida promoters in both species peak during exponential growth then slowly trail off. However, these differences in transcription did not result in more than a 2-fold difference in DksA levels (Fig. 2E). Thus, similar to E. coli, regulation of 54-dependent transcription by ppGpp in P. putida is primarily mediated by changes in ppGpp levels (11) rather than DksA levels (Fig. 2E).

IHF Levels Do Not Restrict 54-transcription in P. putida—Physical interaction of the bEBP and 54-RNAP is aided by IHF-mediated DNA-bending. IHF protein levels increase at the exponential-to-stationary phase growth transition in both E. coli and P. putida and have been shown to be partially under the control of ppGpp in E. coli (26, 36, 37). Therefore, we considered that IHF levels may contribute to ppGpp/DksA mediated control of 54-dependent transcription in P. putida.

To examine this possibility, we first used the same genetic systems as described under Fig. 2 to monitor transcriptional output from the xh-Po(-IHF)/Px series of 54-promoters (Fig. 1) that differ from the xh-Po/Px series only in minimal changes that abolish IHF binding (13). We found that the temporal transcription profiles of the xh-Po/Px series and cognate xh-Po(-IHF)/Px plasmids were indistinguishable from that shown for the Po/Po-luxAB reporter in Fig. 2A, except that maximal transcriptional output was reduced by lack of IHF-binding.
higher (~25-fold) dependence on IHF seen with the xh-Po/Pu promoter is consistent with data from Pu in its native context, which is also highly dependent on IHF both in vitro and in P. putida (Ref. 26 and references therein).

Next we tested the temporal expression profiles of the xh-Po(IHF)/Pu reporters in ppGpp- and DksA-deficient P. putida KT2440 derivatives. Similar to the data for the IHF-binding proficient Po/Po-luxAB (Fig. 2A), deficiency of either of these two regulatory molecules decreased transcription in a similar manner from reporters that lack the capacity to bind IHF (supplemental Fig. S1), suggesting that ppGpp and DksA primarily mediate their effects at other levels. However, because this series of $\sigma^{54}$-promoter exhibited a higher degree of dependence on IHF in P. putida than when assessed in E. coli (13), we were prompted to test if the in vivo levels of P. putida IHF restrict transcription from any of the $\sigma^{54}$-promoters. To this end we generated a derivative of the KT2440::dmpR-Tel strain with an additional copy of the P. putida ihfAB genes under the control of the P$_{\text{pac}}$-promoter. IPTG induction in this strain produces IHF levels in the exponential phase at least as high as those found in the stationary phase of the wild-type counterpart (Fig. 3B, compare lanes 1s and 3e). The activity of the $\sigma^{54}$-promoters under these conditions, however, only differed in the post-exponential phase, with excess IHF giving rise to a modest 1.1–1.3-fold higher maximal expression level with the different promoters (Fig. 3B and data not shown). We conclude from this data that the native post-exponential phase levels of IHF are near saturating for even extremely IHF-dependent $\sigma^{54}$-dependent promoters, and that IHF levels per se are not a limiting factor for $\sigma^{54}$-promoter output during any phase of growth in P. putida.

IHF-mediated Recruitment of $\sigma^{54}$-RNAP Alone Cannot Relieve Tight Exponential Phase Control of P. putida $\sigma^{54}$ Transcription—The high dependence of the Pu promoter on IHF relates to its role in aiding promoter occupancy by $\sigma^{54}$-RNAP. At Pu, IHF-mediated changes in DNA architecture provide a thus far unique $\sigma^{54}$-RNAP recruitment mechanism by providing a DNA architecture that allows simultaneous binding of the two $\alpha$-subunits of $\sigma^{54}$-RNAP to a distally located DNA UP element. This additional DNA binding element thus increases the affinity of the Pu promoter for $\sigma^{54}$-RNAP (38–40) and would be anticipated to impart higher affinity for $\sigma^{54}$-RNAP to any $\sigma^{54}$-promoter.

To test if the $\sigma^{54}$-RNAP recruitment mechanism of Pu was sufficient to allow detectable transcription from other $\sigma^{54}$-pro-

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**FIGURE 2. Growth phase regulation of transcription.** A, growth (closed symbols) and luciferase activity (open symbols) of P. putida harboring pV708 (Po/Po-luxAB) cultured in LB in the presence of 2-methylphenol. Strains: KT2440::dmpR-Tel (squares) and its otherwise isogenic ppGpp$^-$ (PP1::dmpR, circles) and DksA null (PP2::dmpR; up-triangles) derivatives. B and C, immunoblot analysis of DmpR and $\sigma^{54}$ levels in 40 and 20 $\mu$g of soluble protein extracts prepared from cells grown as shown and harvested at the indicated time points. Co., control: P. putida KT2440 $\sigma^{54}$ null mutant that also lacks DmpR. D, growth (closed symbols) and luciferase activity (open symbols) of monocytophin reporter gene fusions on the chromosomes of P. putida (KT2440::Po/Po-luxAB) and E. coli (MG1655::Po/Po-luxAB). Both P. putida (diamonds) and E. coli (down-triangles) strains were cultured at 30 °C in LB. E, immunodetection of DksA in 2 and 4 $\mu$g of soluble protein extracts prepared from the indicated strains cultured under D and harvested at the indicated time points. Specificity of the antibody was confirmed using 4 $\mu$g of soluble protein prepared from cognate null mutant strains (data not shown).

**FIGURE 3. Influence of IHF on transcriptional output from $\sigma^{54}$-promoters.** A, luciferase reporter gene transcription from $\sigma^{54}$-promoters in P. putida KT2440::dmpR-Tel harboring pV7671 to pV7678, cultured in LB in the presence of 2-methylphenol. The data show the peak transcriptional output at the 6-h time point. B, immunodetection of IHF levels and luciferase reporter gene transcription from the xh-Po/Po-luxAB reporter plasmid pV7671 in P. putida KT2440::dmpR-Tel-P$_{\text{pac}}$-ihfAB cultured as under panel A in the absence (down triangles) and presence (diamonds) of 0.5 mM IPTG. Immunodetection with antibodies directed toward P. putida IHF used 40 $\mu$g of soluble protein from P. putida KT2440::dmpR-Tel (lanes 1e and 1s), and KT2440::dmpR-Tel-P$_{\text{pac}}$-ihfAB grown in the absence (lanes 2e and 2s), and in the presence of IPTG (lanes 3e and 3s). Cells were harvested at an$ \text{OD}_{600} = 0.35 (e)$ and 3.5 (s) as indicated by the arrows in panel B. The data show the peak transcriptional output at the 6-h time point.
motors in exponentially growing *P. putida*, we generated the series of xh-Pu/Px promoters, which maintain the UAS binding sites for DmpR in exactly the same location relative to the −24, −12 sequences but have the appropriately positioned Pu UP-element and IHF binding site that is located one helical turn from that in the Pu upstream region (see Fig. 1, upper). However, as shown in Fig. 4A, whereas the xh-Pu/Px derivatives exhibit a modest increase in maximal output in the ppGpp-proficient strain, and increase the expression in the ppGpp-deficient strain as compared with their cognate xh-Po/Px counterparts in the stationary phase, no transcription in the exponential phase could be detected (data not shown). Furthermore, no further increase in maximal transcription levels or exponential phase expression was seen with any of the xh-Pu/Px promoters upon overexpression of IHF using the *P. putida* strain described above (data not shown). Because transcriptional initiation at σ54 promoters requires physical interaction between the bEBP and σ54-RNAP, these results suggest that even with additional IHF and the IHF-dependent σ54-RNAP recruitment device, the levels of DmpR and/or σ54-RNAP are too low for sufficient co-occupancy of the promoters to mediate detectable transcription during the exponential phase of growth.

**Tight Exponential Phase Control in *P. putida***

Conversely, the proposed limitation imposed by the co-occupancy outlined above, we have recently shown that artificial elevation of DmpR protein levels in LB-cultured *P. putida* does allow some σ54-Po output during the exponential phase of growth (14), whereas similar manipulations in *E. coli* do not (13). However, even in *P. putida* expressing excess DmpR, ppGpp synthesis triggered by nutrient depletion still greatly enhanced transcription at the exponential to stationary phase growth transition (14). Hence, whereas DmpR levels are a major limiting factor for σ54-dependent transcription in exponentially growing *P. putida*, σ54-RNAP levels also appear to limit transcription in this phase of growth (14).

To determine whether naturally occurring levels of a bEBP in *P. putida* could allow exponential phase σ54-dependent transcription, we used strains expressing the bEBP XylR, which is highly homologous to DmpR and can efficiently bind and activate transcription from the UASs of *Po* in response to its natural effectors (41, 42). The levels of XylR in *P. putida*, like those of DmpR, increase as cells enter stationary phase (43). However, XylR is naturally produced from its *pWW0* plasmid in higher levels than those of DmpR from *pVII150* (30) and the *P. putida* strains used here maintain XylR levels at ~65% of those from the *pWW0* plasmid in this host (30).

As shown in Fig. 4, B–F, XylR regulation results in higher transcription levels than DmpR in the *P. putida* ppGpp-deficient strain with all the test σ54-promoters. Notably, the additional σ54-RNAP recruitment device of the xh-Pu/Px promoter series both increases the transcription levels in the absence of ppGpp as compared with cognate xh-Po/Px promoters (compare Fig. 4, E and F, with C and D), and results in transcription during the exponential phase of growth (Fig. 4, E and F, and data not shown). These results clearly demonstrate that the IHF-mediated σ54-RNAP recruitment devices is operational in these promoters, and provide evidence that the level of the σ54-RNAP holoenzyme is a critical parameter that can restrict exponential phase σ54 transcription in *P. putida*. However, the results also clearly suggest that given sufficient levels of a bEBP, some σ54-dependent systems could still be expressed during rapid high energy growth conditions in *P. putida*.

**Lack of Rsd or PfrA Does Not Influence the Growth Phase Transition Checkpoint of σ54 Transcription**

The data in the preceding sections are consistent with the previous proposal that ppGpp/DksA in part mediate their effects on σ54-transcription by enhancing the levels of the σ54-RNAP holoenzyme and thus promoter occupancy at the exponential to stationary

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**FIGURE 4. IHF-mediated recruitment of σ54-RNAP and bEBP levels in σ54 transcription.** A, the DmpR-regulated activity of strains harboring pVII61 to pVII64 and pVII89 to pVII92 at the 6-h point where peak activity of the ppGpp− strain is observed. Strains KT2440::dmpR-Tel (gray bars) or its ppGpp5 counterpart PP1::dmpR (white bars) were cultured in LB containing 2 mM of the DmpR effector 2-methylphenol. B, shows the XylR-regulated activity of the same series of plasmids as under panel A at the 4.5-h point where peak activity of the cognate ppGpp− strain is observed. Strains KT2440::xylR-Tel (gray bars) and its isogenic ppGpp5 counterpart PP1::xylR (white bars) were cultured in LB containing 1 mM of the XylR effector 3-methylbenzylalcohol. C–F, luciferase reporter gene transcription (open symbols) during growth (closed symbols) of *P. putida* KT2440::xylR-Tel (squares) and its isogenic ppGpp5 counterpart PP1::xylR (circles) harboring the indicated reporter plasmids when cultured as under panel B.

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phase transition (12, 13, 18). In *E. coli*, the levels of the $\sigma^{70}$-binding protein Rsd are partially under ppGpp control (44, 45), and Rsd can sequester both free $\sigma^{70}$ and actively remove $\sigma^{70}$ from the $\sigma^{70}$-holoenzyme in vitro (46, 47). The finding that overexpression of Rsd in *E. coli* enhances transcription from stress-response promoters dependent on alternative $\sigma$-factors such as $\sigma^A$ and $\sigma^D$ (12, 48, 49) led to the speculation that Rsd may be involved in ppGpp-mediated control of $\sigma^{D^4}$-transcription (13). To test this idea we compared $\sigma^{D^4}$-transcription in wild-type and the Rsd null mutant of *E. coli* using the transcriptional reporter plasmid pVI466 (dmpR-Po-luxAB), and generated a null mutant of the Rsd $E. coli$ homologue PfrA in the KT2440-dmpR-Tel strain. Transcription in the *P. putida* isogenic pair was monitored using the Po/Po-luxAB reporter pV1708. However, lack of these proteins in either organism did not significantly alter temporal or maximal $\sigma^{D^4}$-transcription (supplemental Fig. S2). Hence, we conclude that the physiologically relevant levels of these proteins do not significantly impact $\sigma^{D^4}$-transcription under our test conditions where in vivo ppGpp-mediated effects are prominent (Fig. 2).

Robust Stationary Phase Transcription in *P. putida* Correlates with Reduced Promoter Discrimination by Its $\sigma^{D^4}$-RNAP—When analyzed with *E. coli* $\sigma^{D^4}$-RNAP in gel-shift assays, the affinities of the six Po/Px $\sigma^{D^4}$-promoters shown in Fig. 1 were found to lie in the order Po/PnifH < Po/Pn < Po/Po < Po/PnifH409 < Po/PpglA and Po/PppA (13). This order is maintained in similar assays using purified *P. putida* $\sigma^{D^4}$-RNAP (data not shown). Transcriptional reporter gene assays in *E. coli* revealed an 18-fold difference between the lowest affinity promoter (Po/PnifH) and the highest affinity promoters (Po/PpglA and Po/PppA) (13). This order is maintained in similar assays using purified *P. putida* $\sigma^{D^4}$-RNAP (data not shown). Transcriptional reporter gene assays in *E. coli* revealed an 18-fold difference between the lowest affinity promoter (Po/PnifH) and the highest affinity promoters (Po/PpglA and Po/PppA), which was reproduced in vitro using *E. coli* $\sigma^{D^4}$-RNAP (13). This *E. coli* data contrast our findings in *P. putida* where we observe only an ~3-fold difference between the maximal outputs from the highest and lowest affinity promoters (Fig. 5A). However, similar to *E. coli* (13), all the promoters were dependent on both ppGpp and DksA for efficient output in *P. putida*. Lack of either ppGpp or DksA in *P. putida* leads to 3–7-fold reduced transcription, with the low affinity promoters being notably more dependent on ppGpp and DksA than high affinity promoters (Fig. 5A). This data supports the previous proposal, based on extensive *E. coli* data, that ppGpp and DksA deficiency results in reduced levels of $\sigma^{D^4}$-RNAP, which will most severely affect occupancy of low affinity $\sigma^{D^4}$-promoters (13).

The apparent relatively high stationary phase transcription from even the lowest affinity promoters of the Po/Px series of $\sigma^{D^4}$-promoters in wild-type *P. putida* suggested to us that either (i) the stationary phase levels of $\sigma^{D^4}$-RNAP are sufficiently high to allow frequent activation from even very low affinity promoters, or (ii) that the transcriptional apparatus of *P. putida* is less discriminative of sequence differences in the ~33 to +2 region than its *E. coli* counterpart. To test the latter possibility, we employed multiple round transcription assays with *P. putida*-derived proteins under the same conditions previously used with *E. coli*-derived proteins and that recapitulate the known negative and positive effects of these molecules at $\sigma^{70}$-promoters (13). As was previously found with *E. coli*-derived proteins (13), addition of ppGpp and DksA had little, if any, direct effect on in vitro transcription from any of the promoters (Fig. 5B). Importantly, the *P. putida* in vitro reconstituted system reproduced the in vivo differences in transcriptional output from these promoters in wild-type *P. putida*, namely only an ~3-fold difference between the lowest (Po/PnifH) and highest affinity promoter (Po/PpglA), compare Fig. 5, A and B.

To directly compare the activities of the $\sigma^{D^4}$-holoenzymes of *E. coli* and *P. putida*, we determined transcription from the Po/Po promoter in the presence of a constant level (5 mM) of the *E. coli* or *P. putida* core enzymes and increasing concentrations of the $\sigma^{D^4}$-subunit from each organism. To compare saturation kinetics, data were normalized by setting the plateau values as 1 in each case. The combination of PP core with EC $\sigma^{D^4}$ gave barely detectable transcription under any of the conditions tested (data not shown). Data are the average of two independent experiments ± S.E. D, relative transcription using saturating concentrations of holoenzymes as under panel C. Data are the average of the four plateau values shown in panel C, normalized by setting the transcript levels of EC $\sigma^{D^4}$-RNAP as 1.

![Figure 5](https://example.com/figure5.png)
follows that these differences cannot be attributed to differences in holoenzyme concentrations, but reflect the binding and complex formation of the holoenzyme with the Po promoter DNA. Taken together, the data suggests that ppGpp and DksA regulation of $\sigma^{54}$-RNAP to chip-immobilized double-stranded DNA encompassing the $\sigma^{54}$-promoters Po/PnifH, Po/Po, or Po/PglnA. Background resonance units from a reference well with no coupled DNA were always less than 10%, and were subtracted from the values shown. The results are representative of two independent overlapping titrations. Concentrations refer to nanomolar core RNAP used to reconstitute the holoenzymes with 8-fold excess of $\sigma^{54}$-subunit. The rapid binding of both $\sigma^{54}$-RNAPs was abolished with control DNA in which the $\sim 24$, $\sim 12$ motif of either (Po or PglnA) was destroyed, whereas 600 nm $\sigma^{54}$-subunit from each organism exhibit no specific binding under these conditions (data not shown). B and D, P. putida (B) and E. coli (D) $\sigma^{54}$-RNAP binding response relative to that of/PnifH set as 1. Bars represent the average relative response at all concentrations assayed in A and C with standard errors.

The $P$. putida $\sigma^{54}$-Subunit Confers Reduced Promoter Discrimination via Its DNA Binding Properties—To dissect promoter binding and discrimination by $E$. coli and $P$. putida-derived $\sigma^{54}$-RNAPs, we employed Biacore plasmon resonance analysis of binding to chip-coupled double-stranded DNA encompassing the lowest (Po/PnifH), an intermediate (Po/Po), and the highest affinity (Po/PglnA) promoter, under the same buffer conditions as used in $in vitro$ transcription assays. As illustrated in Fig. 6, dose-dependent responses caused by the $\sigma^{54}$-RNAP holoenzymes from the two organisms differed greatly, with that from $E$. coli exhibiting $>5$-fold difference in binding to the lowest versus highest affinity promoter (Fig. 6D), whereas from $P$. putida exhibited only a $<2$-fold difference (Fig. 6B). This higher binding and reduced discrimination between the different promoters by the $P$. putida holoenzyme mirror that observed in both the $in vivo$ and $in vitro$ transcription assays shown in the preceding section.

It seemed likely that this difference between the holoenzymes from the two organisms would be mediated by the DNA binding properties conferred by the cognate $\sigma^{54}$-subunits. To test this idea we compared the binding of 50 nm native and heterologous $\sigma^{54}$-RNAP holoenzymes to each of the three test $\sigma^{54}$-promoters. As shown in Fig. 7, the characteristic DNA-binding profiles of holoenzymes to the different promoters were essentially reproduced in an organism-specific manner depending primarily on the origin of the $\sigma^{54}$-subunit rather than the core RNAP. For example, note the marked poor binding and slow binding kinetics to both of the two lower affinity promoters (PnifH, dashed line, and Po, dash-dot line) with the $E$. coli holoenzyme (Fig. 7B) or heterologous $\sigma^{54}$-RNAP with $E$. coli $\sigma^{54}$ (Fig. 7C), which are comparatively more rapidly bound by the $P$. putida holoenzyme or heterologous $\sigma^{54}$-RNAP with $P$. putida $\sigma^{54}$ (Fig. 7, A and D). However, the magnitude of the responses seen with the different promoters, which reflects the absolute levels of binding, also suggests that the core RNAP to some extent contributes to the rates and final level of binding that is achieved by each combination such that the native $P$. putida combination binds all the promoters most efficiently.
Concluding Remarks—Orthologs of σ^{54} are widely distributed among different bacteria, but the operons that they control are quite diverse and differ from one organism to another. P. putida strains are nutritional and environmentally very adaptable (50), and have to respond to different external conditions and stimuli than the gut-commensal E. coli. In P. putida and related bacteria, σ^{54}-promoters control a variety of processes that are responsive to rapidly changing environmental cues (3), and are prevalent in controlling expression of auxiliary metabolic pathways, such as those for catabolism of aromatic compounds, that feed into the central metabolism (51). The production of specialized enzymes for such pathways is metabolically expensive, and regulatory devices are needed to ensure that these kinds of pathways are not expressed if more favorable sources of carbon and energy are available. The work presented here demonstrates that P. putida σ^{54}-transcription is very tightly controlled under high energy conditions provided by complex media, and a combination of DNA elements was needed to obtain sufficiently high affinity for σ^{54}-RNAP to carry out any appreciable transcription under these conditions. Conversely, in comparison with E. coli, upon nutrient depletion as cells enter the stationary phase, transcription from even comparatively low affinity promoters is robust and this robust transcription is attributable to the reduced promoter discrimination of low affinity promoters conferred by the DNA binding properties of the P. putida σ^{54}-RNAP holoenzyme. Because aromatic compounds are stress agents even for bacteria capable of using them as carbon sources, expression of the enzymes for their catabolism is interfaced with both central metabolism and stress responses (6). With respect to σ^{52}-transcription, our findings suggest that P. putida has evolved and integrated ppGpp/DksA global regulation of σ^{54}-transcription to provide both extremely tight control during rapid growth with vigorous transcription under nutritional stress. It is perhaps for these reasons that σ^{54}-promoters are frequently found in association with control of auxiliary metabolic pathways in this nutritionally adaptable organism.

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REFERENCES