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Minireview

Integrated regulation in response to aromatic compounds: from signal sensing to attractive behaviour

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Summary

Deciphering the complex interconnecting bacterial responses to the presence of aromatic compounds is required to gain an integrated understanding of how aromatic catabolic processes function in relation to their genome and environmental context. In addition to the properties of the catabolic enzymes themselves, regulatory responses on at least three different levels are important. At a primary level, aromatic compounds control the activity of specific members of many families of transcriptional regulators to direct the expression of the specialized enzymes for their own catabolism. At a second level, dominant global regulation in response to environmental and physiological cues is incorporated to subvert and couple transcription levels to the energy status of the bacteria. Mediators of these global regulatory responses include the alarmone (p)ppGpp, the DNA-bending protein IHF and less well-defined systems that probably sense the energy status through the activity of the electron transport chain. At a third level, aromatic compounds can also impact on catabolic performance by provoking behavioural responses that allow the bacteria to seek out aromatic growth substrates in their environment.

Introduction

Bacteria excel in rapidly adapting to scavenge limiting nutrient supplies and occupying hostile environments. The metabolic diversity and plasticity of bacteria in the face of environmental insults and limitations provide an immense reservoir of exploitable regulatory devices and biochemical activities (Ellis, 2000). Among these are the ability to biodegrade and thus remove a wide variety of natural and man-made aromatic compounds discharged through geochemical cycles, urban and industrial activities (Timmis and Pieper, 1999; Dua *et al*., 2002). Prokaryotes from archaea to eubacteria possess the capacity to biotransform aromatic compounds either as pure culture or in consortia. However, the aerobic aromatic catabolic pathways of pseudomonads (and closely related microorganisms) together with those of their associated plasmids are the most extensively studied workhorses of environmental bioremediation of this abundant class of pollutants. At >6 million bp, *Pseudomonas* genomes are among the largest of the ≈70 bacterial genomes sequenced to date. Consistent with their ability to thrive in diverse environments and execute efficient catabolism of a broad spectrum of carbon sources, *Pseudomonas* genomes contain the highest proportion of regulatory genes observed, and a high proportion of genes dedicated to the catabolism, transport and efflux of organic compounds (Nelson *et al*., 2002).

The genetic make-up in terms of genes encoding the catabolic enzymes, although obviously essential, is far from the whole story with respect to how bacterial aromatic catabolic systems function under the very different conditions presented by diverse natural environments or in bioreactors. Aromatic catabolic pathways, like other catabolic processes, have to function efficiently within the context of the host and be regulated in order to avoid detrimental energy fluxes that would otherwise compromise production, host fitness and survival. Transcriptional regulatory properties of pathway gene expression are critical components through which multiple, and sometimes conflicting, signals presented by complex environments are orchestrated. Both specific and global regulatory impact on promoter output thus places the functioning of aromatic catabolic pathways within the network of host cellular processes (see Fig. 1). This review aims to highlight progress and limitations in our understanding of how information is perceived, processed, and integrated to ultimately control aromatic biodegradative properties and microbial behaviour at three levels. First, how regulators detect and respond to the specific signal of the presence of an aromatic compound(s) pertinent to the function of the genes they control. Secondly, how these regulatory

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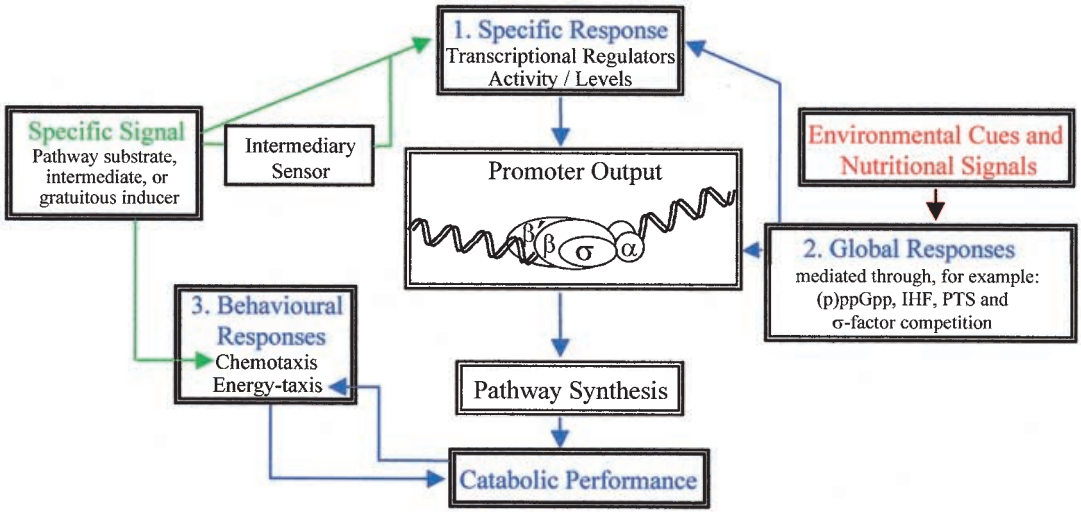

Fig. 1. Schematic illustration of the signals and resulting responses that converge to impact on the catabolic performance of bacteria on aromatic compounds. The specific signal (green) provided by a given aromatic compound controls the activity of the cognate transcriptional regulator to provide the substrate availability 'go' signal for the specific response (1). Environmental and nutritional cues (red) that report on the prevailing conditions modulate different global responses (2) that integrate to 'stop' or subvert the specific regulatory response until the energy status of the host indicates that expression of the specialized aromatic catabolic enzymes will be beneficial. By serving as a chemoattractant or through its energy-generating catabolism, a given aromatic compound can also impact on catabolic performance by provoking a behavioural response (3) that directs bacteria to move towards the carbon source.

circuits are rendered appropriately subservient to, and integrate within, the evolutionary adapted global regulatory systems of the host and, thirdly, how signals are coupled through basal functions to allow beneficial behaviour such as chemo- or energy taxis in response to the information they receive. These aspects are presented in the light of their impact on, and monitoring of, the performance of bacterial biodegradative properties, and in terms of potential improvement of desirable microbial properties.

Natures aromatic sensors

Transcriptional regulators lie at the top of the hierarchy of events that lead to expression of the genes and operons that encode the specialized suites of pathway enzymes for catabolism of aromatic compounds (Fig. 1). Transcriptional control is the key entry point that allows catabolic processes to be expressed only when required and at levels that will provide an adequate metabolic return. Specific proteins that govern aromatic catabolism (some of which are listed in Table 1) include representatives from all major classes of prokaryotic regulators and follow the general molecular mechanisms of the given families to control initiation of transcription (Gerischer, 2002). The specificity of expression of aromatic catabolic pathway genes is built in at the level of transcription through direct modulation of regulator activity by specific substrates or intermediates of the cognate pathway. In the case of two-component systems, the aromatic-sensing function is performed by an intermediary sensory histidine kinase component that communicates the activating signal via phosphotransfer to activate a transcriptional regulatory partner. Hence, a distinguishing feature of many, but not all, regulators of aromatic catabolism is their ability to directly sense the levels of biologically available aromatic pathway substrates. This useful property is harnessed by the incorporation of this class of sensory regulators as the biological component of whole-cell biosensors for monitoring available, and thus biologically relevant, pollution of sites (Keane *et al.*, 2002). Such whole-cell-based biosensors have been developed to detect a whole range of aromatic pollutants (e.g. Hay *et al.*, 2000) and consist of an appropriate sensor regulator–promoter pair in control of a reporter gene with a simply detected phenotype such as bioluminescence or fluorescence. Transport systems that actively bring aromatic compounds into the cell increase the sensitivity of the natural aromatic sensing systems and can be incorporated productively to enhance biosensor performance (e.g. Prieto and Garcia, 1997).

Promiscuity and limitations of aromatic effector recognition

The regulators of aromatic catabolism vary extensively in the number and range of compounds they respond to. For members of the LysR family (Table 1), the activating signal is usually relatively specific, being limited to an intermediate of the corresponding catabolic pathways that is initially produced through basal level transcription (e.g. salicylate

Table 1. Representative regulatory proteins involved in biodegradation of aromatics.

Family	Pathway[a]	Host (plasmid)	Reference
σ^{54}-dependent family			
DmpR	(Methyl)phenols	*Pseudomonas* sp.CF600 (pVI50)	Shingler *et al*. (1993)
HbpR	2-Hydroxybiphenyl	*P. azelaica* HBP1	Jaspers *et al*. (2000)
MopR	Phenol	*Acinetobacter calcoaceticus* NCIB8250	Schirmer *et al*. (1997)
PhlR	Phenol	*P. putida* H (pPGH1)	Burchhardt *et al*. (1997)
PhnR	Phenanthrene/naphthalene	*Burkholderia* sp. RP007	Laurie and Lloyd-Jones (1999)
TbuT	Toluene	*Ralstonia pickettii* PKO1	Byrne and Olsen (1996)
TouR	Toluene	*P. stutzeri* OX1	Arenghi *et al*. (1999)
XylR	Toluene/xylene (upper)	*P. putida* mt-2 (TOL pWW0)	Inouye *et al*. (1988)
Two-component systems			
BpbS/BphT	Biphenyl/PCB	*Rhodococcus* sp. strain M5	Labbe *et al*. (1997)
StyS/StyR	Styrene	*P. putida* CA-3	Velasco *et al*. (1998)
TodS/TodT	Toluene	*P. putida* F1	Lau *et al*. (1997)
AraC/XylS family			
PobC	4-Hydroxybenzoate	*Pseudomonas putida* WCS358	Bertani *et al*. (2001)
XylS	Toluene (*meta*/lower) pathway	*P. putida* mt-2 (TOL pWW0)	Spooner *et al*. (1986)
BenR	Benzoate pathway	*P. putida*	Cowles *et al*. (2000)
LysR family			
CatR	Catechol	*P. putida*	Rothmel *et al*. (1990)
ClcR	Chlorocatechol	*P. putida* (pAC27)	Coco *et al*. (1993)
NahR	Naphthalene/salicylate	*P. putida* (pNAH7)	You *et al*. (1988)
TfdR	2,4-Dichlorophenoxyacetic acid	*Ralstonia eutropha* JMP134 (pJP4)	Leveau and van der Meer (1996)
Examples of cross-regulation within mechanistic groups			
DmpR and XylR cross-regulation of Po and Pu in *P. putida*			Fernández *et al*. (1994)
TbmR and TbuT cross-regulation in *Ralstonia pickettii* PKO1			Leahy *et al*. (1997)
TmoS/TmoT and TodS/TodT from *P. mendocina* and *P. putida*			Ramos-Gonzalez *et al*. (2002)
BenR regulation of the XylS-Pm promoter in *P. putida*			Jeffrey *et al*. (1992)
CatR and ClcR cross-regulation in *P. putida*			Parsek *et al*. (1994)
TfdR and TfdT cross-regulation in *Ralstonia eutropha* JMP134			Leveau and van der Meer (1996)

a. Note that the pathway indicates initial substrate(s) and does not necessarily reflect the effector compound of the cognate regulator as described in the text.
Representative regulators were chosen for their pertinence to the text and to illustrate the range of pathways under consideration. A more extensive list of members of these and other families (including CRP/FNR, GntR, IclR, MarR and TetR families) is given in Diaz and Prieto (2000) and Gerischer (2002).

for NahR, *cis*,*cis*-muconate for CatR and 2-chloro-*cis*,*cis*-muconate for ClcR). Regulators that respond to primary substrates can be quite promiscuous, responding to a whole catalogue of non-metabolizable structural analogues of the pathways they control. Prime examples of this are XylR and DmpR, two mechanistically related σ^{54}-dependent regulators that respond to distinct sets of aromatic effector. XylR, which activates the Pu promoter of the upper operon of pWW0 for conversion of toluene and *m*/*p*-xylene to benzoate and the corresponding alkyl-benzoates, also responds to some quite structurally dissimilar compounds such as chloro- and alkyl-substituted benzyl aldehydes and benzyl alcohols (Abril *et al*., 1989). A somewhat more restricted but still broad response is found with DmpR, the regulator that controls catabolism of phenol, monomethylated phenols and 3,4-dimethylphenol through the Po promoter of pVI150. DmpR can respond to some structural analogues possessing novel chloro or ethyl substituents, but tolerance of chemical variation is markedly dependent on the location of the substituent on the aromatic ring and is generally narrower than for XylR (Shingler and Moore, 1994; Shingler and Pavel, 1995). This class of regulators also includes members that are responsive to biaromatic compounds rather than monoaromatic compounds (e.g. HbpR; Jaspers *et al*., 2000), and some that also additionally respond to non-aromatic pollutants such as trichloroethane (e.g. TbuT; Byrne and Olsen, 1996). Simple sequence comparisons of the sensory regions are not sufficient to deduce the aromatic effector profile as illustrated by MopR (Table 1), the effector profile of which is most similar to DmpR but whose primary sequence is most similar to XylR (Schirmer *et al*., 1997). Although most aromatic-responsive σ^{54}-dependent regulators usually recognize primary substrates, this is not always the case, as exemplified by TouR (Table 1), which is activated by (methyl)phenol intermediates rather than the primary substrates (i.e. toluene and *o*-xylene). This is intriguing in the

light of the idea that, during evolution and pathway assembly, regulation is recruited in an *ad hoc* fashion more on the basis evolutionary history and/or some appropriate regulatory response rather than on the basis of the mechanistic class of transcription activation (de Lorenzo and Pérez-Martín, 1996). Under this scenario, TouR would probably have been adopted to control toluene catabolism by expansion of a (methyl)phenol degradative pathway (Arenghi *et al*., 2001).

Despite promiscuity, aromatic effector recognition by regulators can be of primary importance for catabolic limitations through their poor or non-responsiveness to aromatic compounds that can be dissimulated through cognate pathways. As exemplified by XylS [the regulator of the Pm (lower *meta*-pathway) promoter of pWW0] and DmpR, effector specificity mutants that have removed the regulatory bottleneck by acquiring novel or enhanced ability to respond to given compounds can thus enhance catabolic performance (Ramos *et al*., 1986; 1987; Pavel *et al*., 1994; Sarand *et al*., 2001). The plasticity of aromatic responsiveness is not only observed in the laboratory. For *Pseudomonas* CF600 harbouring pVI150, poor recognition of 4-methylphenol by DmpR limits biodegradative capacity as it elicits suboptimal expression of the enzymes necessary for its catabolism. As little as 2–4 days in 4-methylphenol-contaminated soil results in a 'fitter' mutant subpopulation that can degrade 4-methylphenol more efficiently by the sole virtue of sensory mutations of the DmpR regulator (Sarand *et al*., 2001). Hence, it is likely that selection of mutations that alter effector responses to a given pollutant(s) is a continual and ongoing major adaptation mechanism adopted by microorganisms for optimizing aromatic catabolism in contaminated environments. The distinct domain structure of the sensory component in XylR/DmpR-like proteins (see below) may facilitate fast adaptation and account for the abundance of the XylR/DmpR-like proteins found to control aromatic catabolic pathways.

Aromatic effectors in action

Cross-talk between structurally related regulators in response to distinct aromatic effectors and non-cognate promoters of aromatic catabolism can occur when the pathways are naturally present within the same organism or when artificially introduced (Table 1). This kind of cross-regulation has been exploited to clone regulators of a suspected mechanistic class (e.g. Leahy *et al*., 1997) and to understand the differential regulation of systems that can occur in response to the presence of specific aromatic compounds (e.g. Kessler *et al*., 1994; Cowles *et al*., 2000). Mutations that alter aromatic effector responses of regulators have been central in both identifying regions and domains of proteins that interact with the aromatic effector to result in transcription activation and isolating variants with desired responses. I expand on just a few representative studies here for the LysR, AraC/XylS and XylR/DmpR σ^{54}-dependent families that have been studied most extensively in this respect. Schematic representations of the distinct functional regions of these proteins are illustrated in Fig. 2.

The LysR-type regulators are usually bound to DNA irrespective of the presence or absence of the effector ligand and share a high degree of similarity in their N-terminal helix–turn–helix (HTH) DNA-binding domains. A poorly conserved central region separates the DNA-binding region of the protein from the C-terminal region that is involved in multimerization and transcriptional activation. The divergence of this central region probably reflects structural variations to accommodate the variety of inducers that activate this family of proteins (Schell, 1993). This suggestion has gained strong support from polymerase chain reaction (PCR)-generated mutant analysis of the aromatic-responsive NahR representative of this family, in which enhanced affinity and/or novel effector responses were traced to residues located within a 60-amino-acid stretch of the central region (Cebolla *et al*., 1997). RNA polymerase recruitment by relieving the DNA bending caused by the regulator in the absence of effector appears to be the underlying mechanism of how effector binding translates to activation of transcription by this family of proteins. Interestingly, fine tuning of the induction pathway by the LysR-type CatR, to avoid superfluous and energetically wasteful production of pathway enzymes, has been identified. This mechanism involves a low-affinity CatR binding site located downstream of the transcriptional start of the catabolic operon that becomes occupied only when concentrations of the CatR-activating pathway intermediate *cis*,*cis*-muconate become high, thus signalling high metabolic flow. Binding of CatR to the low-affinity

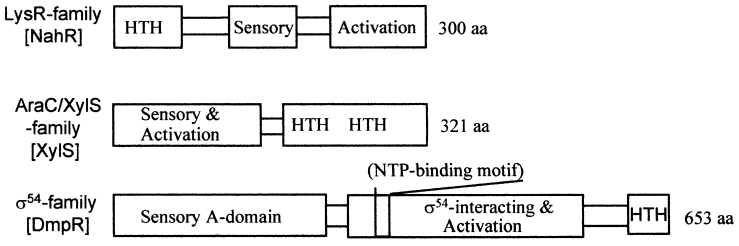

Fig. 2. Schematic representation of the functional regions of transcriptional activators of aromatic catabolism discussed in the text. HTH stands for helix–turn–helix motif and indicates the involvement in DNA binding. Sensory indicates regions identified as interacting with the activating effector compound, while activation indicates regions involved in promoting transcription from cognate promoters.

binding site impairs transcriptional activation from the cognate catabolic promoter, which lowers gene expression after sufficient enzyme levels for efficient substrate utilization have been attained (Chugani *et al*., 1998; Tover *et al*., 2000).

XylS is the most extensively characterized aromatic-responsive member of the AraC/XylS family of regulators. Regulators of this protein family typically possess an ≈100-amino-acid C-terminal region sufficient for both DNA binding at tandem repeats and transcriptional activation. In most cases, this region is linked to a non-conserved N-terminal region involved in regulating activity (Tobes and Ramos, 2002). Such a separable domain structure for XylS has recently been rigorously confirmed (Kaldalu *et al*., 2000). XylS is expressed from two promoters, Ps1 and Ps2 (Gallegos *et al*., 1996). XylS-dependent transcription from the Pm promoter is only (alkyl)benzoate dependent at the low cellular XylS concentrations that are constitutively produced from the Ps2 promoter. At the high levels of XylS produced by the action of XylR on the Ps1 promoter, XylS can promote transcription from Pm even in the absence of its (alkyl)benzoate-activating signal. Thus, for this protein, both effector modulation of the dynamics of transition between an active and inactive configuration and modulation of its cellular levels are involved in its regulatory properties. Extensive mutagenesis of the *xylS* gene has been performed to identify effector-interacting regions and has pinpointed an 11-amino-acid glycine-rich N-terminal patch that is likely to be involved in effector recognition, although mutations that alter the effector specificity and dependence map throughout the protein (Ramos *et al*., 1986; Michan *et al*., 1992a). Mutation in the N-terminus can restore effector control that has been lost as a result of a mutation in the C-terminus, strongly indicating that the two functionally distinct parts of XylS interact to attenuate its DNA-binding properties and that effector control is mediated through regulation of this interaction (Michan *et al*., 1992b). However, it remains to be determined whether binding of the aromatic effector simply relieves constraints to give a larger pool of the active conformation of the protein, or whether the aromatic effector has a more active role in stimulating the DNA-binding activity of the C-terminal region (Kaldalu *et al*., 2000).

The σ^{54}-dependent family of transcriptional activators are mechanoenzymes that use ATP hydrolysis to trigger remodelling of σ^{54}–RNA polymerase intersubunit and DNA interactions actively to promote transcriptional initiation (Zhang *et al*., 2002). Regulators of this family exhibit a complex domain structure but bear in common a central σ^{54} interaction module that is also known as an EBP module (for enhancer-binding protein) as these proteins typically act from binding sites located unusually distant from the promoters that they control. The central σ^{54} interaction module of these regulators is usually linked to a DNA-binding domain at its C-terminus and to a regulatory domain containing one or more sensory motifs at its N-terminus (Studholme and Dixon, 2003). For the XylR/DmpR-like subgroup, this sensory domain (A-domain) is sufficient to bind the aromatic effector directly and, at least in the case of DmpR, a single site is used for the response to multiple aromatic effectors (O'Neill *et al*., 1998; 1999). Many different methods, including genetic selections (Delgado and Ramos, 1994; Shingler and Pavel, 1995), PCR-generated mutations (Wise and Kuske, 2000), DNA shuffling (Skärfstad *et al*., 2000; Garmendia *et al*., 2001) and *in situ* selection (Sarand *et al*., 2001), have identified structural variants with novel response properties. In these regulatory systems, the sensory A-domain serves to represses the innate transcriptional activating property of the central domain through specific interdomain interactions that are broken upon aromatic effector binding (Fernández *et al*., 1995; Pérez-Martín and de Lorenzo, 1995a; Shingler and Pavel, 1995; Ng *et al*., 1996). A small structured linker that joins the sensory A-domain to the σ^{54} interaction module in DmpR, XylR and many other aromatic-responsive members is intimately involved in co-ordinating this aromatic effector response (Garmendia and de Lorenzo, 2000; O'Neill *et al*., 2001). Interestingly, acquisition of novel response properties does not necessarily involve the creation of a new effector-binding property, but rather appears to lie within the ability of effector binding to productively couple to transcriptional activation (O'Neill *et al*., 1999). Some mutations that alter and weaken the normally strong repressive interdomain interaction lead to both novel response properties and also affect the normally strict effector control of transcription, resulting in some level of transcription even in the absence of effectors (Ng *et al*., 1996; Garmendia *et al*., 2001). Thus, both aromatic effector binding to the sensory A-domain and co-ordinated consequent release of interdomain repression are required for transcriptional activation. The DNA-binding properties of one member of this family, HpbR, has recently been shown to couple to, and be stimulated by, aromatic effector binding (Tropel and van der Meer, 2002). Ultimate control, however, is dictated by the ability of aromatic effector binding to translate to a conformation that allows the regulator to adopt its ATP binding-triggered multimeric state that is central for transcriptional activation *per se* (Wikstrom *et al*., 2001). Intriguingly, the effector-bound form of the A-domain may play a role in stabilizing the active multimeric subunit conformation of DmpR, suggesting an active role for the A-domain in both repression and stimulation of the transcriptional promoting property of this class of proteins (Wikstrom *et al*., 2001). Most recently, a purely bioinformatics-based structural model for the sensory A-domain component has been developed. This model goes a long way to

explain the experimental phenotypes observed with sensory A-domain mutations and may serve as a basis for more rational design of desired aromatic responsiveness of this class of proteins (Devos *et al*., 2002).

Integration within host physiology

Microbes of the same environmental niche are under tough competition for available resources and have to perceive and integrate multiple signals pertaining to a variety of stresses including limited nutrient availability and physicochemical stresses such as oxygen and water tension, pH, ambient temperature and, sometimes, the toxicity of a whole assortment of (co)pollutants. The ability to adapt readily to using the most energetically favourable available nutrients under the prevailing conditions signalled by these multiple parameters thus provides a competitive advantage. The production of whole pathways (typically 10–20 specialized enzymes) imposes a metabolic load that only confers an advantage under conditions in which the aromatic compound is available. Therefore, retention of these catabolic systems, whether newly acquired or long-standing chromosomal or plasmid-encoded systems, is only truly advantageous if their usefulness does not compromise the host fitness under other conditions. Thus, where studied, expression of aromatic catabolic systems is usually subservient to global regulatory input that signals the nutritional and energy status of the host (see Fig. 1) to downregulate production of the specialized enzymes until they are required. The appreciation that coupling to such dominant global regulatory input will be needed to fully integrate metabolic engineering to host microbial physiology under working conditions provides a practical impediment to understand the underlying mechanisms (Cases and de Lorenzo, 1998; Timmis and Pieper, 1999; Diaz and Prieto, 2000). With reference to aromatic catabolic systems, what better ways are there to integrate them than adopting those naturally incorporated into aromatic-responsive regulatory systems?

With the notable major exception of carbon catabolite repression, global regulatory factors of pseudomonads and related bacteria have been found to follow the general paradigms developed for *Escherichia coli*. Below, I emphasize global systems in which the impact has been studied with respect to aromatic degradative systems, but the mechanisms uncovered have general applicability to the regulation of many processes. These global systems function through the normal channels of transcription control, namely (i) through the cellular levels of the different forms of holoenzyme RNA polymerase; (ii) through promoter architecture; and (iii) through the activity of transcriptional regulators. However, it should be noted that post-transcriptional mechanisms that operate through small regulatory RNAs have an enormous regulatory potential to control both specific and global responses (reviewed by Wagner and Vogel, 2003). Furthermore, small regulatory RNA has been found to be involved in the control of carbon flow in *E. coli* (e.g. Pernestig *et al*., 2003), and analogous systems are likely to feature in regulation in pseudomonads.

Sigma factors, stress and competition for the right to transcribe

The group of seven σ factors of *E. coli* is supplemented in pseudomonads such as *Pseudomonas putida* and *Pseudomonas aeruginosa* by an additional 17 ECF (extracytoplasmic function) σs that, in all probability, contribute to the exquisite adaptability of these organisms, in particular to combat iron limitation (Martinez-Bueno *et al*., 2002). The different σ factors programme the catalytic core RNA polymerase (E, subunit composition $\alpha_2\beta\beta'\omega$) to engage and initiate transcription at the distinct sets of promoters within the prokaryotic genome. Competition between the alternative σ factors for limiting core RNA polymerase thus creates global transcriptional hierarchies through the resulting levels of the different alternative holoenzymes that are available for each class of promoters (Farewell *et al*., 1998; Ishihama, 2000; Jishage *et al*., 2002). The levels of most alternative σ factors vary in response to specific signals. However, the structurally distinct σ^{54} is expressed at ≈15–20% of the levels of the house-hold σ^{70} in *E. coli* and, like expression of σ^{70}, the levels of σ^{54} are constant throughout the growth curves of *E. coli* and *P. putida* (Cases *et al*., 1996; Ishihama, 2000).

Modulation of the differing pools of alternative holoenzymes by stress imposed by the presence of aromatic compounds or other environmental signals interweaves the promoters of aromatic catabolic genes to central physiological control. For example, the expression profiles of the heat shock σ^H factor and the stationary phase σ^S factor have been integrated within the XylS-Pm regulatory system to provide the option of continual transcription from Pm throughout the growth curve of *P. putida* (Marqués *et al*., 1999). In this capacity, (alkyl)benzoate, in addition to its role as the activating signal of XylS, also acts as a stress signal leading to upregulation of σ^H and utilization of $E\sigma^H$ at Pm during exponential growth, which then switches to utilization of $E\sigma^S$ when σ^S becomes available at the onset of stationary phase. The mechanism responsible for (alkyl)benzoate upregulation of σ^H in *P. putida* is unknown but does not act through *rpoH* transcription (Manzanera *et al*., 2001).

The converse is found in many regulatory systems, in which rapid growth and high-energy conditions render the system essentially mute until growth slows down at the transition to stationary phase. This transition occurs transiently and crudely mimics the 'hungry response' that opti-

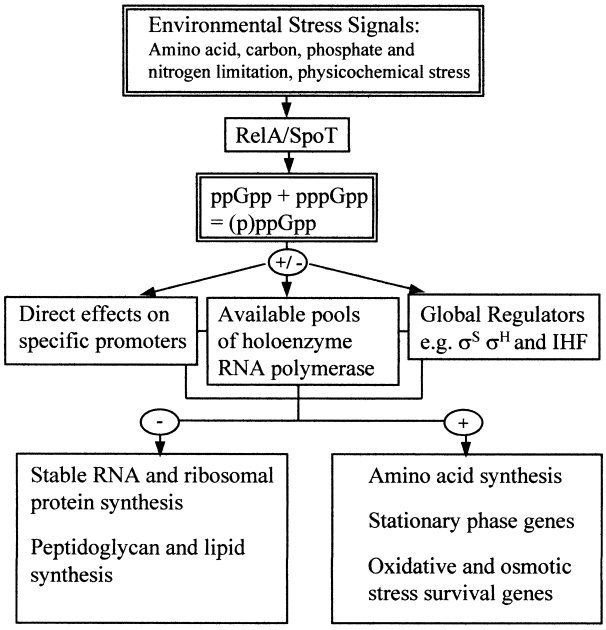

Fig. 3. Overview of the signals and global consequences of the (p)ppGpp regulon. Stress, signalled through availability of nutrients and physicochemical cues, stimulates the synthetase activities of RelA and SpoT, leading to increased levels of the alarmones ppGpp and pppGpp. The (p)ppGpp signal, through its action on RNA polymerase, results in either positive (+) or negative (–) effects on the output from individual promoters, some of which control the expression of proteins that are, in themselves, global regulators. In addition, (p)ppGpp also modulates competition in favour of alternative σ factors for limiting core and, thus, the relative abundance of the available pools of the different holoenzyme RNA polymerases. The net effect of these global adaptations is to downregulate (–) processes associated with rapid growth and to upregulate (+) the expression of genes that serve to optimize nutritional scavenging and stress survival. The scheme is based on information primarily gleaned from *E. coli* but, where tested, has been found to be valid for *P. putida*.

mizes scavenging capabilities and is quite different from the response to nutrient excess or complete starvation (Ferenci, 1999). A specific case is the σ^{54}-dependent DmpR-Po regulatory system, in which restricted transcription under high-energy conditions and coupling to host physiology appears to be solely attributable to the alarmone (p)ppGpp that heralds nutritional and physicochemical stress (Sze and Shingler, 1999; see also Fig. 3). The (methyl)phenol-responsive DmpR-Po regulatory circuit is kept in check by low levels of (p)ppGpp that are elicited by rapid growth on rich media and are released to give high levels of transcription when high levels of (p)ppGpp are produced in response to the hunger signals generated at the exponential to stationary phase transition point or during growth on poor carbon sources (Sze *et al*., 1996; Sze and Shingler, 1999). In both *E. coli* and *P. putida* strains lacking RelA and SpoT [the (p)ppGpp synthetase I and II proteins], transcription from the σ^{54}-dependent Po promoter is severely inhibited despite the constant levels of DmpR and σ^{54} in both strains (Sze *et al*., 2002). Conversely, artificially elevated levels of (p)ppGpp (produced by overexpression of RelA) in *P. putida* allow functioning of the DmpR-Po regulatory circuit even under normally non-permissive high-energy conditions (Sze and Shingler, 1999). A critical key to this regulatory phenomenon lies not in a requirement for (p)ppGpp *per se*, but rather in the central role of (p)ppGpp in dictating the outcome of competition between the house-keeping σ^{70} and σ^{54} for limiting core RNA polymerase (Laurie *et al*., 2003). The same mechanism also operates for σ^{S} and σ^{H} (p)ppGpp-dependent promoters, and a direct positive effect of (p)ppGpp to favour σ^{H} competition for core has been documented *in vitro* (Jishage *et al*., 2002). In all these systems, mutations that render σ^{70} less able to compete for core RNA polymerase in *in vitro* and *in vivo* assays also restore transcription in strains unable to produce (p)ppGpp. Consistently, mild artificial underproduction or sequestering of σ^{70} both restores transcription in the absence of (p)ppGpp and, in the case of the σ^{54}-dependent DmpR-Po circuit, allows transcription during exponential growth on rich media in which (p)ppGpp levels are low and the Po promoter is normally silent (Laurie *et al*., 2003). Thus, (p)ppGpp provides a mechanism that alters the relative competitiveness of σ^{54} and other alternative σ factors to meet cellular demands during nutritional and physiological stress. However, it remains to be determined whether (p)ppGpp enhances the binding of alternative σ factors or decreases the binding of σ^{70} or, alternatively, operates at some other level such as σ factor exchange.

The properties of constant comparatively high levels and high affinity of σ^{54}, together with (p)ppGpp stimulation of its otherwise poor competitive ability against σ^{70}, provides a mechanism for rapid alteration in occupancy and transcription of σ^{54}-dependent promoters in response to changes in the environment without *de novo* σ synthesis (Laurie *et al*., 2003). In addition to the involvement of σ^{54} in the control of catabolism of many aromatic compounds in *P. putida* (Table 1), σ^{54} is also involved in controlling many carbon catabolic systems in *P. aeruginosa* (e.g. Nishijyo *et al*., 2001). Hence, this regulatory mechanism has the potential to have a far-reaching impact on the differential use of carbon sources in addition to involvement in nitrogen regulation.

The (p)ppGpp network

As illustrated in Fig. 3, (p)ppGpp is probably the most far-reaching bacterial global signalling molecule through both its direct affects mediated by binding at the interface of the β and β′ subunits of RNA polymerase and consequent modulation of transcription from specific promoters and, indirectly, through regulatory cascades as a result of its stimulatory effects on promoters that control other global regulators (Chatterji and Ojha, 2001). For example,

(p)ppGpp directly stimulates transcription from the σ^{54}-dependent Po and Pu promoters with purified components *in vitro* (Carmona *et al*., 2000; Laurie *et al*., 2003). In a side-by-side *in vitro* comparison, the stimulatory effect of (p)ppGpp was markedly higher on Po than on the Pu promoter when activated by a constitutively active form of XylR (Carmona *et al*., 2000). These direct effects, observed in the absence of σ factor competition, would thus be amplified *in vivo* by the (p)ppGpp-mediated superimposed regulation of available pools of $E\sigma^{54}$ as described above. Direct comparison of the *in vivo* impact of loss of (p)ppGpp likewise showed that the XylR-Pu system was markedly less affected than the DmpR-Po system (Sze *et al*., 2002). Intriguingly, although the behaviour of these two systems in (p)ppGpp-proficient and -deficient *E. coli* roughly mimics the observations in (p)ppGpp-proficient and -deficient *P. putida*, the physiological responses in the two hosts differ (Sze *et al*., 2002). First, (p)ppGpp-deficient *E. coli* are rendered auxotrophic, whereas (p)ppGpp-deficient *P. putida* remain prototrophic. Secondly, lack of (p)ppGpp has somewhat less impact on these regulatory systems in *P. putida* than in *E. coli*. These observations suggest differences in the thresholds of the (p)ppGpp regulatory networks of the two bacteria. In *E. coli*, (p)ppGpp is intimately involved in governing the downregulation of superfluous translational capacity through the stringent promoters for ribosomal proteins and stable RNAs (rRNAs and tRNAs), and presumably performs the same function in *P. putida*. The codon usage of genes involved in the translational capacity of *P. putida* KT2440 have been noted to differ substantially from those used by typical *P. putida* genes. It has been suggested that consequent utilization of separate tRNA pools might aid metabolic versatility by uncoupling global protein synthesis from synthesis of the components of the translational machinery (Weinel *et al*., 2002). Utilization of separate tRNA pools would also be predicted to influence both the levels and the temporal synthesis of (p)ppGpp production as ribosome-associated RelA is only triggered to produce (p)ppGpp when uncharged tRNAs enter the ribosomal A box. Thus, with respect to (p)ppGpp, subtly differing 'hunger' signals in the form of uncharged tRNAs may, at least in part, account for the differences observed between (p)ppGpp-impacted systems in *E. coli* and *P. putida*.

To add further complexity to the story, optimal performance of both DmpR-Po and XylR-Pu systems require IHF (integration host factor) (de Lorenzo *et al*., 1991; Sze *et al*., 2001). The levels of this protein are regulated by (p)ppGpp, at least in *E. coli*, and expression is upregulated upon entry into stationary phase in both *E. coli* and *P. putida* (Aviv *et al*., 1994; Valls *et al*., 2002). This site-specific DNA-bending protein is required by a large number of σ^{54}-dependent promoters for its architectural role to bring the distally bound regulators in close physical proximity to the promoter-bound $E\sigma^{54}$. However, IHF impacts many promoter types and processes and is thus a global regulator in itself (Arfin *et al*., 2000). In addition to its role in providing close physical contact, IHF serves two other functions at the Pu promoter. First, it serves as a 'restrictor' to prevent promiscuous cross-activation by other σ^{54}-dependent regulators (Pérez-Martín and de Lorenzo, 1995b). Secondly, IHF helps to recruit $E\sigma^{54}$ to the Pu promoter by providing a promoter architecture that allows interaction of the α-subunit of RNA polymerase with a distally located 'UP-like' DNA element that is otherwise out of reach (Bertoni *et al*., 1998; Carmona *et al*., 1999). This recruitment role for IHF, which greatly facilitates output from the Pu promoter, is not observed for the Po promoter, which is less dependent on IHF (Sze *et al*., 2001). Although IHF does not serve a $E\sigma^{54}$ recruitment role at the Po promoter, it does have a distinct role in promoting or stabilizing open complexes of $E\sigma^{54}$ at Po by an as yet unresolved mechanism (Sze *et al*., 2001). The *in vivo* occupation of the IHF site of Pu in response to the changing levels of IHF plays a major role in restricting the capacity of the Pu promoter during exponential growth. However, additional signals are also incorporated at the exponential to stationary phase transition (Valls *et al*., 2002). Thus, for the two very similar aromatic-responsive DmpR-Po and XylR-Pu pairs, differing impacts of IHF, direct effects of (p)ppGpp on promoter kinetics and (p)ppGpp regulation of available $E\sigma^{54}$ pools probably differentially combine to lock the functioning of both these promoters to the 'hungry' conditions encountered at the exponential to stationary phase transition.

Inhibitory metabolites and carbon catabolite repression

Carbon catabolite repression is a frequently observed phenomenon in the regulation of aromatic catabolic pathways of pseudomonads, although little is known of the mediators or mechanisms underlying repression in most cases. This term has been used to describe both the preferred use of one carbon source over another, leading to biphasic (diauxic) growth, and milder repression that results in downregulation but not complete prevention of transcription from a promoter by growth in the presence of an additional carbon source. The mechanisms used by pseudomonads are likely to be very different from the cAMP-CRP-dependent glucose repression paradigm of *E. coli* for three main reasons. First, in contrast to enterics that thrive on glucose and other sugars, acetate and various tricarboxylic acid (TCA) cycle intermediates are the preferred carbon source for pseudomonads, and it is these compounds rather than glucose that are most frequently found to exert the most severe repression (reviewed by Collier *et al*., 1996). Secondly, cAMP levels

do not vary appreciably with different carbon sources in these bacteria (Collier *et al*., 1996) and, thirdly, even though the pseudomonad CRP homologue, Vfr, is a global regulator, its loss does not have any effect on succinate-mediated carbon catabolite repression of three systems tested (Suh *et al*., 2002), and thus far Vfr has not been found to impact on the regulation of aromatic catabolism. The Crc protein of pseudomonads, which mediates cAMP-independent repression of some catabolic pathways by an as yet unresolved mechanism (Collier *et al*., 1996), has likewise not yet been reported to be involved in the regulation of catabolism of any aromatic compound.

The end-point compounds produced from the specialized catabolic pathways for catabolism of aromatic compounds (e.g. succinate, pyruvate and succinyl- and acetyl-CoA) feed into the TCA cycle. Thus, a simple way to monitor and integrate within host metabolic activity would be to sense the state of the TCA cycle. The ClcR LysR-type regulator of chlorocatechol catabolism provides a conceptually pleasing example of how this can be achieved simply and directly through the modification of regulator activity (McFall *et al*., 1997; 1998). In this regulatory system, ClcR is activated by the pathway intermediate 2-chloro-*cis*,*cis*-muconate, and transcriptional fusion studies have shown that growth of the cells on the TCA intermediates succinate, citrate or fumarate results in inhibition of ClcR-dependent transcription. Fumarate specifically and reversibly inhibits 2-chloro-*cis*,*cis*-muconate induction of ClcR in *in vitro* transcription assays. Thus, it appears that it is fumarate alone of the TCA cycle intermediates that is directly sensed to mediate repression by its ability to compete with 2-chloro-*cis*,*cis*-muconate for the same binding site on ClcR. Transcription by the closely related CatR regulator that is activated by *cis*,*cis*-muconate is unaffected by the addition of fumarate (McFall *et al*., 1998), and it is as yet unclear how far reaching this simple but effective mechanism will turn out to be.

As mentioned above, the molecular mechanisms of catabolite repression observed on transcription of genes for other aromatic catabolic pathways are largely unknown, and many pieces of the puzzle are missing. However, some clues are provided by the following studies. Carbon sources such as succinate, glucose or gluconate have been found to downregulate the σ^{54}-dependent transcription mediated by the XylR-Pu-regulated circuit *in P. putida* (Duetz *et al*., 1994; Holtel *et al*., 1994; Cases *et al*., 1999). In the case of succinate catabolite repression, continuous culture experiments demonstrated that strong repression is only found during non-limiting growth on succinate, and that this repression is released under succinate-limiting conditions. These findings, together with the observation that non-carbon growth-limiting conditions such as phosphate and nitrogen limitation could mimic strong succinate catabolite repression, suggest that the energy status of the cell could play a key role, although it has yet to be resolved how this is mediated (Duetz *et al*., 1996). Glucose catabolite repression of Pu transcription, which results in a lower level of repression of about two- to threefold, has been found to relate to the action of the *ptsN* and *ptsO* genes. These two genes are clustered along with the *rpoN* (σ^{54}) gene in *P. putida* and encode components for an alternative phospho*enol*pyruvate:sugar phosphotransferase system (PTS) (Cases *et al*., 1999; 2001a). Glucose repression through this PTS link is genetically separable from the factors that lock Pu expression to the post-exponential phase in rich media, suggesting that it intercepts the XylR-Pu regulatory circuit by a different route (Cases and de Lorenzo, 2000). Furthermore, two-dimensional gel electrophoresis of a *P. putida ptsN* mutant has demonstrated global affects on protein expression that do not involve all σ^{54}-dependent promoters and are not limited to σ^{54}-dependent genes (Cases *et al*., 2001b). Moreover, this PTS–glucose repression link is not manifested on the σ^{54}-dependent DmpR-Po system (Sze *et al*., 2002), so a general connection through σ^{54} appears to be unlikely. Hence, although very much open to question, it is possible that this PTS may link through some glucose/energy-sensing system.

A possible link to energy sensing has also been suggested by two recent genetic studies that used transposon mutagenesis strategies to identify components involved in signalling catabolite repression on two mechanistically very different regulatory systems. The first of these is a σ^{54}-dependent regulatory circuit that controls a phenol catabolic pathway of the pPGH1 plasmid of *P. putida* strain H, which is highly homologous to the plasmid-encoded DmpR-Po system (Petruschka *et al*., 2001). The second involves the LuxR family-type regulator AlkS that controls the alkane degradation pathway of the OCT plasmid of *P. putida* GPo1 (Dinamarca *et al*., 2002). In both these studies, multiple mutants that could partially counteract succinate repression were found to map within the *P. putida cyoABCDE* cluster that encodes cytochrome *o* ubiquinol oxidase, the main terminal oxidase of the electron transport chain under high-energy conditions. These results suggest that an early and general step in the signal transduction process may involve energy sensing by monitoring the flow of electrons through the transport chain or through the redox status of the cell (Petruschka *et al*., 2001; Dinamarca *et al*., 2002).

Interweaving into host physiology

As illustrated by the work described on the mechanistically related pairs DmpR/XylR and CatR/ClcR, cross-talk between structurally related regulators can be exploited to help to identify and probe differential interweaving into host physiology. In the case of the DmpR/XylR regulatory

pair, not only can the regulators cross-activate each other's promoters, but the sensory A-domains can be exchanged to give fully functioning proteins that respond to the discrete effector profile of the parent protein (Shingler and Moore, 1994; Garmendia *et al*., 2001). This property can be very useful in pointing out new directions to understand integration via different mechanisms about which little is known. An example of this concerns the ATP-dependent FtsH protease, of which a counterpart exists in pseudomonads. In *E. coli*, FtsH is essential for aerobic survival, and the levels of FtsH are controlled in response to physiological cues (Qu *et al*., 1996). In an FtsH$^-$ *E. coli* strain, XylR-mediated transcription from Pu is drastically reduced, as is transcription mediated by three other σ^{54}-dependent regulators tested, namely NtrC, NifA and PspF (Carmona and de Lorenzo, 1999). By analogy with the role of FtsH in controlling σ^H activity, this finding spurred the idea that FtsH may also be essential for Eσ^{54} activity through its action on σ^{54} or a putative antisigma factor. However, subsequent work has demonstrated that this is unlikely as the σ^{54}-dependent DmpR-Po system is fully independent of FtsH. Cross-regulation studies also showed that FtsH dependence of the XylR-Pu circuit is attributable to properties of the regulator rather than the promoter (Sze *et al*., 2002). Intriguingly, simple possession of the A-domain of DmpR on the body of XylR renders the functioning of this chimera completely independent of FtsH (E. Skärfstad and V. Shingler, unpublished), suggesting that some other unknown, but perhaps chaperone-like, activity of FtsH is at play (Sze *et al*., 2002).

The studies discussed in the preceding sections illustrate that control of aromatic degradative abilities can be interwoven to connect with host physiology in a number of different ways and, even mechanistically, very similar systems can be integrated by disparate means. All the information so far available indicates that it really does not matter exactly how evolution has operated to integrate a given system as long as it does the job of subduing specific pathways until their products are needed (Cases and de Lorenzo, 2001). Ignorance of the complete mechanistic picture of known proteins and signals and their levels under different conditions, combined with the large potential global regulatory impact of the hefty proportion of genes of unknown function, severely limits our understanding of the complete picture. To date, most of our more detailed understanding of how integration within host physiology is achieved has come from the study of a few archetypal systems. It is therefore likely that these studies only scratch the surface of a myriad of mechanisms that can differentially impact a given system and come together to severely or partially dampen transcription under conditions in which expression of the specialized pathway enzymes is non-beneficial. Hence, many additional novel and interesting mechanisms to achieve the same end are likely to be discovered in the years to come.

Seeking out substrates and a higher energy state

Efficient removal of aromatic contaminants from sites can potentially be enhanced by the ability of bacteria to recognize and swim towards the aromatic compound, thus preventing mass transfer limitations that impede biodegradation. In addition, the ability to relocate to favourable oxygen tension conditions would also be anticipated to enhance catabolic performance of aerobic aromatic degradative pathways. A number of chemotactic responses to the presence of aromatic compounds have been reported to be induced co-ordinately with genes for their catabolism, suggesting an integrated bacterial strategy to move towards and degrade aromatic compounds (reviewed by Pandey and Jain, 2002; Parales and Harwood, 2002). As detailed in the following sections, some of these behavioural responses may use energy-sensing mechanisms akin to those involved in global regulation.

The ability of bacteria to move non-randomly towards an attractive compound (or away from an obnoxious one) is probably mechanistically the best understood bacterial behavioural response. Bacterial chemotaxis has been studied most extensively with enteric bacteria in response to simple sugars, amino acids and organic acids that are sensed outside the cell by a suite of ligand-binding *trans*-membrane receptors, MCPs (methyl-accepting chemotaxis proteins). The MCPs transmit the signal in the form of conformational changes to the histidine autokinase CheA, which serves as the phosphodonor for cognate response regulators that reset the membrane receptors for the next round of sensing and bind to the flagella motor to control the swimming behaviour of bacteria. The chemotactic machinery of some bacteria involves additional MCPs and chemotaxis proteins. For example, *P. putida* encodes 25 or more MCPs compared with five for *E. coli* (Parales and Harwood, 2002). However, regardless of added complexity, the fundamental processes of signal detection and transmission are probably conserved (Armitage and Schmitt, 1997).

A number of chemotactic responses to aromatic compounds have been reported that are induced directly in response to the presence of an aromatic compound itself. However, in only a few cases has the sensory component been identified. Induction of aromatic chemotactic responses, like those of the activity of transcriptional regulatory proteins, can be promiscuous and include compounds that cannot be degraded by cognate pathway(s). A recent example of this is found with *P. putida* F1, in which toluene induces a chemotactic response to a wide range of compounds including some that cannot be catab-

olized by the cognate *tod*-encoded pathway (Parales *et al*., 2000). In this system, mutants defective in toluene catabolism by virtue of inactivation of pathway genes retain the chemotactic response profile, while inactivation of the TodS/TodT regulatory two-component system abolishes the chemotactic response. Thus, the inducible chemotactic response appears to be instigated by toluene rather than a pathway metabolite and is co-regulated through the TodS/TodT sensor–regulator pair controlling expression of the pathway enzymes. However, the sensory component that allows coupling to the basal chemotactic machinery has yet to be identified (Parales *et al*., 2000).

In the few cases in which an aromatic sensory component has been found, it is co-transcribed along with the genes required for the catabolism of the aromatic compound. This is the case for the MCP-like NahY sensory component for chemotaxis of *P. putida* towards naphthalene that is encoded at the end of the *meta*-cleavage operon of pNAH7 and thus regulated by NahR (Grimm and Harwood, 1999). Inactivation of NahY prevents chemotaxis towards, but not growth on, naphthalene, suggesting that it is the sensor that couples directly to control chemotactic behaviour. Two other apparent chemotactic aromatic sensor proteins, PcaK of *P. putida* and TfdK of the pJP4 plasmid of *Ralstonia eutropha* JMP134, are likewise co-transcribed with genes for catabolism of the inducing molecule (Harwood *et al*., 1994; Hawkins and Harwood, 2002). PcaK and TfdK, rather than being classic MCPs, are non-essential importers of 4-hydroxybenzoate and the herbicide 2,4-dichlorophenoxyacetate, respectively, which belong to the major facilitator superfamily of transport proteins. How binding or passage of aromatic compounds through these transporters links mechanistically to chemotactic behaviour is as yet unknown. However, it may involve conformational signalling to a membrane-associated or intracellular MCP or other proteins that signal to the basal chemotactic machinery. Although no direct evidence exists, the implication from these few studies is that transporters of aromatic compounds may play an important role for signal-specific detection and transduction in order to direct bacteria towards aromatic compounds. In this respect, it is pertinent to note that motile *P. putida* KT2440, which encodes the potential to degrade a large range of aromatic compounds (Jimenez *et al*., 2002), likewise has multiple putative homologues of PcaK (Nelson *et al*., 2002).

Signal-specific chemotaxis responses such as those described above are typified by three properties, namely (i) they can be elicited by non-metabolizable analogues; (ii) the chemotactic response is unaffected by mutations within genes encoding catabolism of the compound; and (iii) the specific signal remains a chemoattractant even in the presence of metabolizable compounds. In direct contrast to the metabolism-independent chemotaxis behaviour described above, some chemotaxis responses in bacteria require the metabolism of the effector molecule. For example, a strict correlation between the ability of *Ralstonia* sp. strain SJ98 to degrade and be attracted to nitroaromatic compounds has been reported (Samanta *et al*., 2000). This type of behavioural response, which is used by a wide range of bacterial species for taxis towards diverse molecules, shares signalling pathways in common with energy taxis responses such as aero-, photo- and redox taxis (Alexandre and Zhulin, 2001). In these systems, the signal for the behavioural response originates within the electron transport chain, and aerotaxis uses the Aer receptors that sense redox changes through the N-terminal FAD-binding PAS domain. These changes are transmitted to the MCP-like C-terminal domain and subsequently transduced to CheA (Taylor *et al*., 2001). Within metabolism/energy-dependent chemotaxis, bacteria do not sense the compound *per se*, rather sensing is thought to be achieved through detection of change in the cellular energy status through mechanisms analogous to those of Aer. The genome of *P. putida* KT2440 contains a number of genes with a domain architecture similar to that encoding the Aer receptor. Taxis of *P. putida* KT2440 expressing the DmpR-controlled *dmp* pathway towards (methyl)phenols has recently been found to fulfil all the criteria to be mediated by an energy taxis mechanism (I. Sarand and V. Shingler, in preparation). First, only (methyl)phenol aromatic compounds that can be metabolized by the pathway elicit the behavioural response; gratuitous inducers of the transcriptional regulator DmpR do not. Secondly, simple expression of the whole suite of the *dmp* pathway genes from a heterologous promoter results in an analogous behavioural response profile. Finally, expression of a subpart of the *dmp* pathway to restrict the capacity of the pathway to the metabolism of just phenol likewise restricts the taxis response profile to phenol alone. Thus, it appears likely that *P. putida* KT2440 possesses energy-sensing receptor(s) that couple catabolism of phenol to the basal chemotactic machinery. This observation suggests the idea that efficient catabolism might provoke energy taxis of *P. putida* KT2440 towards any aromatic compound that can be metabolized to allow the bacteria to seek out a higher energy state.

Concluding comments

Direct sensing of aromatic substrates is clearly achieved through many mechanisms that elicit different responses ranging from precise control of the activity of a transcriptional regulator through to the manifestation of chemotactic behavioural responses that would benefit the bacteria in their quest for a more comfortable niche. The studies described above also emphasize that, in choosing or

designing microbes for aromatic biodegradative purposes, many parameters are worthy of consideration. In addition to catabolic capacity, it is important to consider both specific and global regulatory control that impact on optimization of metabolic flow and also the behavioural characteristics, such as chemo- and energy taxis, of bacteria in response to both aromatic substrates and gratuitous inducers. The implication of energy-sensing mechanisms, about which very little is known in pseudomonads, in both carbon catabolite repression and behavioural responses raises a number of interesting questions concerning how these systems are interconnected and whether they share receptors or mechanisms in common. These questions and redressing the limited knowledge about global regulation and catabolite repression in pseudomonads certainly merit attention.

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