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Specific and Global Regulation of Genes Associated with the Degradation of Aromatic Compounds in Bacteria

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Abstract

A large number of bacteria are able to degrade aromatic carbon sources employing different strategies. All these pathways are objects of regulatory control at the level of gene expression. This includes specific control in response to the availability of the respective substrate and in many cases global control responding to other available carbon sources or to the metabolic status of the cell. Here, the regulatory proteins responsible for gene regulation are reviewed in particular in correlation to other proteins with a similar primary structure. Most common is the appearance of regulators of the LysR family; other abundant regulator types are NtrC/XylR-type proteins, AraC/XylS-type proteins and the IclR-type proteins. Almost all of the regulators exert their effects as activators of gene expression with the exception of the GntR-type proteins, which are exclusively described as repressors. Factors involved in individual cases of global regulatory mechanisms are enterobacterial CAP, (p)ppGpp, Crc protein, and direct modification of a specific regulator. However, for most pathways of aromatic compound degradation, the molecular mechanisms causing global regulation are not understood.

Introduction

Pathways for the catabolism of aromatic compounds play a crucial role in the mineralization of organic matter since a considerable part of plant-derived biomass consists of lignin. Lignin is a high-molecular compound which consists of irregularly connected aromatic monomers. Thus, it is not surprising to find enzymatic routes capable of feeding the diverse aromatic compounds resulting from the initial depolymerization accomplished by fungi into the central carbon catabolic pathways in a wide range of bacteria (Harwood and Parales, 1996; Smith, 1990; van der Meer, 1997; Williams and Sayers, 1994). Cleavage of the aromatic part is accomplished by dioxygenases in the presence of oxygen, or by reduction and hydrolysis via the benzoyl-CoA pathway under anaerobic conditions (Harwood and Gibson, 1997). As in all specialized bacterial pathways, enzymes for the degradation of aromatic compounds are only synthesized when the respective substrates are present, and thus, all pathways characterized so far underlie specific gene regulation. Further, in many cases (if not in most cases) they are also subject to global regulatory control.

Here the different regulator proteins used for the control of these pathways will be reviewed. They will be grouped according to their primary structural similarities. Since genome sequence information is becoming available at an increasing rate, the numbers of presumptive proteins identified based on their similarity to characterized proteins is increasing with the same speed. In this communication, I focus on regulatory proteins that have been described based on experimental evidence. The goal is not completeness but to structure the many described regulator proteins according to similarity, and to compare members in these groups with respect to functional similarities. Finally a glimpse of the current knowledge about superimposed levels of regulation will be provided.

Proteins Governing Expression of Genes for Conversion of Aromatic Carbon Sources Belong to Distinct Families of Regulatory Proteins

Comparison of newly described regulator proteins often leads to identification of similarity with other proteins or already described families of proteins. These similarities on the amino acid sequence level are in many cases correlated with a similar domain organization and a similar functional mechanism (Sander and Schneider, 1991). In the following paragraphs, regulatory proteins classified according to their recognized similarity are introduced. An overview of these proteins is given in Table 1. Figure 1 gives a sketch of functional domains of the four most common groups of regulator proteins.

The AraC/XylS Group

A limited number of regulators of aromatic degradative pathways shows homology to AraC from *Escherichia coli* (Greenblatt and Schleif, 1971; Miyada *et al.*, 1980). AraC was the first member of this group to be identified and was studied in great detail. Three DNA binding sites have been described for this regulator and they are exceptional in the sense, that they don't contain inverted repeats, but direct repeats. The

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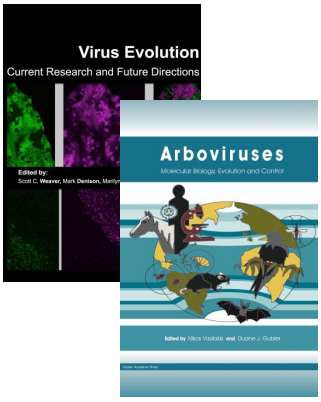

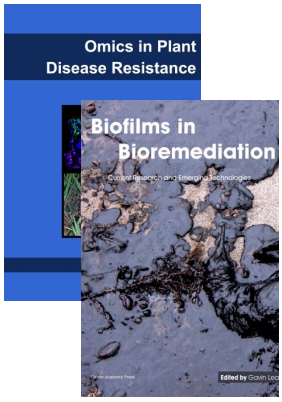

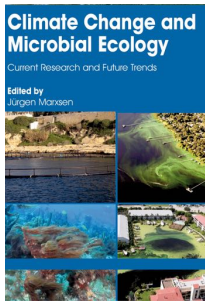

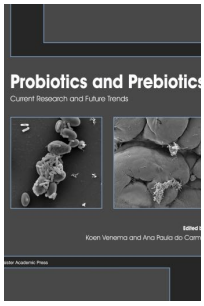

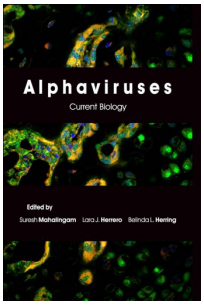

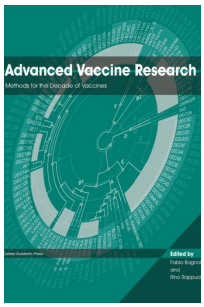

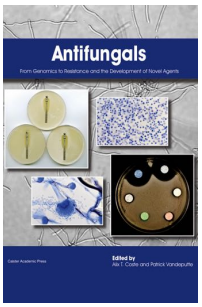

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Table 1. Overview of regulatory proteins involved in control of degradation of aromatic compounds.

Regulator	Organism	Regulated gene(s)/pathway	Mode of action[1]	Reference
AraC/XylS group				
BenR	*Pseudomonas putida*	benzoate degradation via *meta* cleavage plasmid-borne pathway, *ortho* ring fission, POB uptake (indirect?)	activator	(Cowles *et al.*, 2000)
HpaA	*E. coli* W	*hpaBC*, 4-hydroxyphenylacetate catabolism	activator	(Prieto *et al.*, 1996; Prieto and Garcia, 1997)
PobC	*Pseudomonas putida* WCS358	*pobA*, *p*-hydroxybenzoate hydroxylase	activator	(Bertani *et al.*, 2001)
PobR	*Agrobacterium tumefaciens*	*pobA*, *p*-hydroxybenzoate hydroxylase	activator	(Parke, 1997)
PobR	*Pseudomonas* sp. strain HR199	*pobA*, *p*-hydroxybenzoate hydroxylase?	?	(Overhage *et al.*, 1999)
XylS	*Pseudomonas putida* TOL plasmid	*meta* cleavage	activator	(Ramos *et al.*, 1997)
NtrC/XylR group				
AreR	*Acinetobacter* sp. strain ADP1	*areCBA*, degradation of benzylalkanoates	activator	(Jones and Williams, 2001)
DmpR	*Pseudomonas* sp. CF600, plasmid pVI150	(methyl)phenol catabolic operon	activator	(Shingler *et al.*, 1993)
DmpR	*Thauera aromatica*	anaerobic phenol degradation?	?	(Breinig *et al.*, 2000)
MopR	*Acinetobacter calcoaceticus* NCIB8250	*mop* operon, multicomponent phenol-hydroxylase	activator	(Schirmer *et al.*, 1997)
PhlR	*Pseudomonas putida* strain H	phenol degradation	activator	(Burchhardt *et al.*, 1997)
TbuT	*Ralstonia pickettii* PKO1	*tbu* regulon (catabolism of toluene, benzene and related alkylaromatic hydrocarbons)	activator	(Kahng *et al.*, 2000)
XylR	*Pseudomonas putida* TOL plasmid	upper pathway genes (toluene → benzoate or subst. compounds)	activator	(Ramos *et al.*, 1997)
LysR group				
BenM	*Acinetobacter* sp. strain ADP1	*benABC, benD, catA*, benzoate and catechol degradation	activator	(Collier *et al.*, 1998)
CatM	*Acinetobacter* sp. strain ADP1	*catA, catBCDIJF*, catechol degradation	activator	(Romero-Arroyo *et al.*, 1995)
CatR	*Pseudomonas putida*	*catBCA, pheB,A*, catechol and phenol degradation	activator	(Parsek *et al.*, 1994)
CatR	*Pseudomonas putida* biotype (ATCC 12633)	*catB, catA*, catechol degradation	activator (?)	(Houghton *et al.*, 1995)
CbnR	*Ralstonia eutropha* NH9	*cbn* genes, 3-chlorocatechol degradation	activator	(Ogawa *et al.*, 1999)
ClcR	*Pseudomonas putida*	*clcABD*, chlorocatechol degradation	activation	(Parsek *et al.*, 1994)
HcaR	*Escherichia coli* K12	*hcaA1A2CD, hcaB*, 3-phenylpropionic acid degradation	activator	(Diaz *et al.*, 1998)
PcaQ	*Agrobacterium tumefaciens*	*pcaDCHGB*, protocatechuate degradation	activator	(Parke, 1996a)
PcaQ	*Pseudomonas* sp. strain HR199	*pca* genes, protocatechuate degradation?	?	(Overhage *et al.*, 1999)
PcaQ	*Sagittula stellata* E-37	*pca* genes?	?	(Buchan *et al.*, 2000)
PcaQ	*Roseobacter* group, isolate Y3F	*pca* genes?	?	(Buchan *et al.*, 2000)
SalR	*Acinetobacter* sp. strain ADP1	*salA*, salicylate hydroxylase	activator	(Jones *et al.*, 2000)
SalR	*Pseudomonas putida* S-1	*sal* genes, salicylate degradation	activator	(Sato *et al.*, 2001)
TcbR	*Pseudomonas* sp. strain P51	*tcbCDEF*, chlorocatechol oxidative operon	activator	(van der Meer *et al.*, 1991)
TfdR	*Pseudomonas putida*	*tfdCB*, 2,4-dichlorophenoxyacetic acid degradation	activator	(Vedler *et al.*, 2000)
TfdR	*Ralstonia eutropha* JMP134	*tfdCDEF*, 2,4-dichlorophenoxyacetic acid and chlorocatechol degradation	activator	(Leveau and van der Meer, 1996)
IclR group				
CatR	*Rhodococcus opacus* 1CP	*cat*, catechol degradation (?)	?	(Eulberg and Schlömann, 1998)
HppR	*Rhodococcus globerulus* PWD1	3-(3-hydroxyphenyl)propionate degradation (?)	?	(Barnes *et al.*, 1997)
MhpR	*Escherichia coli* K12	3-(3-hydroxyphenyl)propionate degradation	activator	(Ferrandez *et al.*, 1997)
OhbR	*Pseudomonas aeruginosa* 142	oxygenolytic ortho dehalogenation of halobenzoates (?)	?	(Tsoi *et al.*, 1999)
ORF3	*Agrobacterium radiobacter* S2	protocatechuate degradation (?)	?	(Contzen and Stolz, 2000)
PcaR	*Pseudomonas putida*	*pcaK, pcaF, pcaIJ, pcaR*, protocatechuate degradation	activator	(Guo and Houghton, 1999)
PcaR	*Agrobacterium tumefaciens*	*pcaI,J,* protocatechuate degradation (?)[2]	?[2]	(Parke, 1997)
PcaR	*Rhodococcus opacus* 1CP	*pca*, protocatechuate degradation (?)	?	(Eulberg *et al.*, 1998)
PcaU	*Acinetobacter* sp. ADP1	*pcaIJFBDKCHGquiBCXA*, protocatechuate degradation	activator/ repressor	(Gerischer *et al.*, 1998; Trautwein and Gerischer, 2001)
PobR	*Acinetobacter* sp. ADP1	*pobA*, *p*-hydroxybenzoate hydroxylase	activator	(DiMarco *et al.*, 1993; DiMarco and Ornston, 1994)
MarR group				
BadR	*Rhodopseudomonas palustris*	*badDEFG*, anaerobic benzoate degradation	activator	(Egland and Harwood, 1999)
HpaR	*Escherichia coli* W	4-hydroxyphenylacetic acid	?	(Prieto *et al.*, 1996)
HpcR	*E. coli* C	homoprotocatechuate degradation	repressor	(Roper *et al.*, 1993)

Table 1. Continued

2-component systems				
StyS/StyR	*Pseudomonas putida* CA-3, Y2	*styABCD*, styrene(r)phenylacetate	activation	(Velasco *et al.*, 1998)
TodS/ TodT	*Pseudomonas putida* F1	*tod* gene cluster, toluene degradation	activation	(Lau *et al.*, 1997)
TutB/TutC	*Thauera aromatica*	toluene degradation	?	(Coschigano and Young, 1997)
GntR group				
AphS	*Comamonas testosteroni* TA441	multicomponent phenol hydroxylase	repressor	(Arai *et al.*, 1999)
CymR	*Pseudomonas putida* F1	*p*-cymene ⇒ *p*-cumate	repressor	(Eaton, 1997)
PaaX	*Escherichia coli* K12	*paa*, phenylacetic acid degradation	repressor	(Ferrandez *et al.*, 2000)
PhcS	*Comamonas testosteroni* R5	multicomponent phenol hydroxylase	repressor	(Teramoto *et al.*, 2001)
VanR	*Acinetobacter* sp. strain ADP1	*vanAB*	repressor	(Morawski *et al.*, 2000)
FNR/CRP group				
AadR	*Rhodopseudomonas palustris*	anaerobic *p*-hydroxybenzoate degradation	activator	(Dispensa *et al.*, 1992) (Egland and Harwood, 1999)
HbaR	*Rhodopseudomonas palustris*	anaerobic *p*-hydroxybenzoate degradation	activator	(Egland and Harwood, 2000)

[1] Many of the regulatory proteins have a repressing effect on the expression of their own genes, this effect is not included.
[2] The respective quality is assumed, but not proofen.

affinity of AraC to these sites changes dependent on the presence of the inducer arabinose. Binding to two of the DNA targets, intervening DNA forms a loop and in this situation transcription is repressed. In the presence of the inducer the affinity of AraC is higher to another pair of adjacent DNA binding sites and transcription becomes induced. Proteins in this group have a stretch of 99 amino acid residues in common with an overall similarity of more than 20%. In most of the proteins this conserved region is located at the C-terminal end of the regulators. Secondary structure analysis predicts two potential α-helix-turn-α-helix (HTH) DNA binding domains in this region. This, together with analysis of mutant proteins in this region, suggests the function of DNA binding for this domain. The low solubility of many members of this family is the reason for the scant biochemical basis for these predictions (Gallegos *et al.*, 1997). The XylS protein was first identified on the basis of its gene on the *Pseudomonas putida* plasmid pWWO (TOL plasmid) (Inouye *et al.*, 1986). It is certainly the best-characterized regulator involved in regulation of expression of aromatic degradation enzymes within

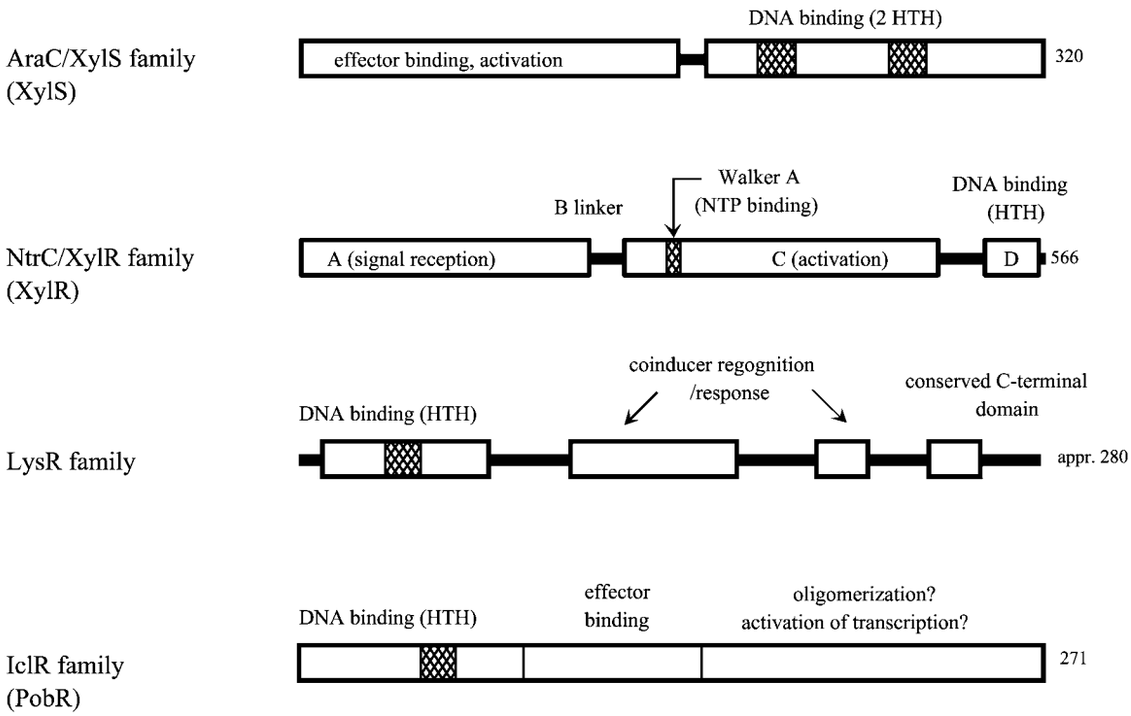

Figure 1. Functional domains of the most abundant regulator types given for a well characterized representative protein or as a summary of several family members as indicated.

this group. XylS is required for the expression of the so-called *meta*-pathway operon, encoding genes for the conversion of alkylbenzoates into Krebs cycle intermediates (Assinder and Williams, 1990). Residues of this protein responsible for effector binding (substituted benzoates) have been identified. These results point to a function of the amino-terminal and central regions in effector binding suggesting distinct domains within the protein with different function (Ramos *et al.*, 1990). The domain structure has been confirmed by mutational analysis revealing two functional domains which probably interact with each other (Kaldalu *et al.*, 2000; Manzanera *et al.*, 2000). Two binding sites for XylS upstream of the regulated promoter Pm were identified between –45 and –49 and between –56 and –70 by systematic mutagenesis of the respective area (González-Pérez *et al.*, 1999) and by DNase I footprinting (Kaldalu *et al.*, 1996). The covered DNA includes a tandem of 15 bp homologous direct repeats. The direct repeat arrangement of the binding site and the mode of DNA binding of XylS are reminiscent of the arrangement of DNA binding sites and the DNA contact pattern of AraC protein from *E. coli*. Transcription at Pm is independent of the sigma factor σ^{70}, instead RNA polymerase with σ^{32} or σ^{38} drives expression (Marques *et al.*, 1999). Residues in the C-terminal domain of the α subunit of RNA polymerase (α -CTD) have been identified to be crucial for the XylS-RNA polymerase interaction (Ruiz *et al.*, 2001). Expression of *xylS* is driven by two promoters. A complex interplay between XylR (a NtrC regulator), the XylR effector, IHF protein and RNA polymerase with sigma factors σ^{70} or σ^{54} insures the right level of the regulator for an efficient and immediate response to changes in the offered carbon source (Marques *et al.*, 1998).

Two regulators activating the expression of *p*-hydroxybenzoate hydroxylase (PobA) in *Agrobacterium tumefaciens* (PobR)(Parke, 1997) and strain WCS358 of *P. putida* (PobC)(Bertani *et al.*, 2001) have been assigned to the AraC/XylS family. The first description of PobC revealed its function in *pobA* gene expression in response to *p*-hydroxybenzoate and to a lesser extent to protocatechuate; PobR from *A. tumefaciens* responds to *p*-hydroxybenzoate. The presence of putative *pobR* genes with similarity to the AraC/XylS group in the genomes of other bacteria indicates a common use of an AraC-type regulator for the regulation of expression of *pobA* genes (*Azotobacter chroococcum*, *Rhizobium leguminosarum*, *Pseudomonas* sp. strain HR199, Table 1). The only exception is *Acinetobacter* sp. strain ADP1 PobR, which is an IclR-type protein (DiMarco *et al.*, 1993). Recently, a chromosomally encoded XylS homologue (62% identity) was described in *P. putida* with multiple activities (Cowles *et al.*, 2000). The BenR protein activates expression of the chromosomal *benABC* genes in response to the effector benzoate. In addition, it activates expression of the TOL plasmid encoded *meta*-cleavage pathway operon. This explains the long-standing observation that the Pm promoter of the *meta*-cleavage pathway remained inducible by benzoate in the absence of XylS (Cuskey and Sprenkle, 1988). The *benABC* promoter region contains a direct repeat sequence highly similar to the XylS binding site upstream of Pm. A third effect of BenR is the benzoate-mediated repression of *p*-hydroxybenzoate uptake by repressing the *pcaK* expression. PcaK is a transporter of *p*-hydroxybenzoate (Nichols and Harwood, 1995). This latter effect is probably indirect since it could not be observed in an *E. coli* background. As in the case of PobR discussed above, the regulator BenM from *Acinetobacter* sp. strain ADP1 controlling the expression of genes for benzoate breakdown, which are highly homologous to the respective *P. putida* genes, does not belong to the AraC family; it is a LysR-type regulator.

Degradation of aromatic compounds has also been observed in a number of *E. coli* strains, including *E. coli* K12. The catabolism of 4-hydroxyphenylacetate via a *meta*-cleavage pathway in *E. coli* W has probably been recruited as a catabolic cassette. The expression of the respective genes is governed by the AraC-type positive regulator HpaA as shown for the PBC promoter upstream of *hpaBC* genes for the initial hydroxylase. Two characteristic direct repeats were observed in the upstream region of PBC (Prieto and Garcia, 1997).

The NtrC/XylR Group

Regulators within the NtrC/XylR group are controlling promoters that are recognized by RNA polymerase with the alternative σ factor σ^{54}. NtrC/XylR regulators concerned with aromatic compound degradation form a subgroup within the σ^{54}-dependent regulators. They sense and respond to the presence of effector molecules whereas many members of the σ^{54}-dependent regulator family are part of two component systems (Shingler, 1996). Functions controlled by σ^{54}-dependent regulators are not essential for the cells. RNA polymerase associated with σ^{54} binds to the characteristic –12/–24 promoters (Thöny and Hennecke, 1989) but is unable to form open transcriptional complexes. The latter is made possible by the σ^{54}-dependent regulator and the hydrolysis of bound ATP is believed to provide the necessary energy for the process (Weiss *et al.*, 1991). Another characteristic quality of σ^{54}-dependent regulators is that their binding site is usually located 100 to 200 bp upstream of the regulated promoter which led to the terms upstream activating sequences (UAS) or enhancer-like sequences for these binding sites. The intervening DNA has been shown to loop out. This process is aided in several cases by the DNA-bending quality of integration host factor (IHF) or intrinsic DNA curvature (Pérez-Martún *et al.*, 1994). Despite the long distance between RNA polymerase and the regulator bound to the DNA, the orientation on the helix relative to each other is critical. σ^{54}-dependent regulators display a distinct domain structure. Three separate domains exert different functions in effector sensing (A domain), ATP binding

and hydrolysis (C domain) or DNA binding (D domain) (see Figure 1) (North *et al*., 1993). The DNA binding domain always contains a HTH motif. As detailed below, a working model for the NtrC/XylR subgroup is an interdomain repression of the ATP-hydrolyzing activity by the sensing domain in the absence of effector. This repression is relieved after effector binding (Shingler, 1996). Two regulators within the NtrC/XylR subgroup have been analyzed intensely by a long number of fascinating investigations: the *Pseudomonas* regulators XylR and DmpR. XylR encoded on the TOL plasmid pWWO from *P. putida* activates transcription from the so called Pu promoter. This promoter controls the synthesis of upper pathway enzymes that transform *m*-xylene into *m*-methylbenzoate (Ramos *et al*., 1997). XylR also stimulates expression of *xylS* from the σ^{54}-dependent Ps1 promoter without effecting expression from the constitutive Ps2 promoter (Gallegos *et al*., 1996). Third XylR represses its own synthesis (Bertoni *et al*., 1998). Due to these multiple activities XylR is referred to as master regulator controlling TOL plasmid catabolic pathway expression (Ramos *et al*., 1997). The regulator is activated by a wide variety of aromatics with different functional groups (Delgado and Ramos, 1994). The XylR binding sites have been characterized thoroughly (Perez-Martin and de Lorenzo, 1996b). The consensus sequence is an inverted repeat, of which two are found upstream of the regulated promoters. They are centered around –135 and –165 upstream of Pu and around –146 and –176 upstream of Ps1. As observed for the relative orientation of regulator and RNA polymerase on the helix, the relative orientation of XylR bound to the two binding sites to each other is also important. Offsetting the binding sites by 5 bp (which corresponds to 0.5 helix turns) considerably decreased promoter activity in vivo (Abril and Ramos, 1993). Recently, the interdomain B linker connecting the A domain and the C domain could be demonstrated to have an important influence on effector binding (Garmendia and de Lorenzo, 2000).

Like the plasmid-borne XylR, DmpR was found to be the gene product of a plasmid-encoded pathway. The *dmpR* gene is part of the *dmp* operon on megaplasmid pVI150 in *Pseudomonas* sp. strain CF600 enabling this organism to grow on the expense of different phenols. After an initial hydroxylation, the aromatic compounds feed into a *meta*-cleavage pathway. The subregion responsible for effector binding within the signal reception domain A of DmpR has recently been identified by DNA shuffling between DmpR and XylR (Skarfstad *et al*., 2000). The capabilities of bacteria to adapt to certain contaminated soils by enhancing degradative capacities was shown to be caused by a mutational change within the effector binding subregion (Sarand *et al*., 2001). The contribution of IHF has been documented and furthermore these experiments revealed a stimulation at the DmpR-dependent Po promoter in the absence of the DmpR binding sites by IHF (Sze *et al*., 2001). The linker between the sensing and the ATP-binding domain (B linker) was originally thought of as a flexible linker. Recently it has been shown that this short region does have a function in repression of the ATPase activity of the C domain. These findings refute the existing model that the level of the ATPase activity directly controls the level of transcription. Rather the function of the A domain is suggested to be the control of transcriptional activation by hindering productive interaction with the σ^{54}-transcriptional apparatus in the absence of effectors (O'Neill *et al*., 2001). Regulators similar in amino acid sequence and function have been found in other bacteria like plasmid-encoded PhlR from *P. putida* strain H (Burchhardt *et al*., 1997), AphR from *Comamonas testosteroni* (Arai *et al*., 1998), PhhR from *P. putida* P35X (Ng *et al*., 1995) and MopR from *Acinetobacter calcoaceticus* NCIB8250 (Schirmer *et al*., 1997). The latter protein regulates the expression of a multicomponent phenol hydroxylase; the product catechol is subsequently processed through an *ortho*-cleavage pathway. MopR differs from DmpR in its individual effector profile. Repression of MopR activity by simultaneous expression of the isolated A domain confirmed observations made with XylR of the negative transdominance over the native regulator (Perez-Martin and de Lorenzo, 1996a; Schirmer *et al*., 1997). AreR was identified in *Acinetobacter* sp. strain ADP1 and suggested to control growth on benzyl alkanoates mediated by the *araCBA* genes (Jones and Williams, 2001). A number of different benzyl alkanoates induce the expression and the AreR sequence shows all the characteristics of other well-described NtrC/XylS family members. An inverted repeat sequence was found 74 bp upstream of the typical σ^{54}-dependent promoter sequence and was suggested to be the binding site for AreR. Strains with dysfunctional *areR* or *rpoN* genes are disturbed in the induction of *araCBA* expression. Another example of a characterized NtrC/XylR group member with slightly different qualities was found in *Ralstonia pickettii* PKO1 (Byrne and Olsen, 1996). TbuT governs metabolism of benzene and toluene via a chromosomally encoded pathway ultimately feeding into a *meta*-fission pathway. The spectrum of this regulators' effectors includes the nonaromatic pollutant trichloroethene. Another unusual quality is the common transcription of the structural genes and the regulator gene within one operon and the resulting cascade expression. A regulator in this group deviating somewhat from XylR and DmpR is HbpR from *Pseudomonas azalaica* HP1 (Jaspers *et al*., 2000). It is the key regulator for 2-hydroxybiphenyl metabolism and this compound and a few similar chemicals are active as effectors. Thus, HbpR is the first regulator within this subclass that recognizes biaromatic rather than monoaromatic compounds. A single amino acid exchange within the A domain locked HbpR in a constitutively active form. Last but not least, the NtrC/XylR group has a representative in the anaerobic world. The gram-negative β-proteobacterium *Thauera aromatica* was isolated under denitrifying conditions with the substrate phenol which it degrades after an initial carboxylation via the benzoyl-CoA pathway (Harwood *et al*., 1999). The available information is limited to the sequence and

location of the gene and the typical –12/–24 consensus promoter sequence upstream of the first structural gene. Since there is no growth with phenol in the presence of oxygen, an additional O_2-dependent regulation is suggested.

LysR Family Members in the Regulation of Catabolic Pathways for Aromatic Compounds

A large number of regulators has been identified with closest relationship to the family typified by the LysR protein (Schell, 1993). This very abundant group of regulators displays highest homology in the N-terminus, which contains a HTH-motif supplying the DNA binding function. The other functions transcriptional activation, effector binding, and multimerization reside in the C-terminal portion of the proteins and are less well defined in most cases. All regulators of the LysR family are activators of expression of genes encoding enzymes that are associated with *ortho*-cleavage through the β-ketoadipate pathway. The best-characterized examples are probably ClcR and CatR from *P. putida* (Parsek *et al.*, 1994) regulating the initial steps of catechol and 3-chlorocatechol conversion respectively. Inducers are the products of the first cleavage steps (*cic,cis*-muconate and its chlorinated derivative). Having bound the effector molecules, the DNA segments covered by these two regulators are very similar. Bending of the DNA by the bound regulators is suggested to play a role in the regulatory process. Thorough analysis revealed the contribution of three distinct binding sites of CatR to regulation, one of which has a repressing function (McFall *et al.*, 1998). Both proteins have been shown to interact with the α-CTD of RNA polymerase. CatR controls a second operon, the *pheA,B* genes encoding enzymes for the introduction of phenol into the β-ketoadipate pathway and it has been shown that the CatR-mediated activation of this operon is very similar to its regulatory action upstream of *catBCA* (Tover *et al.*, 2000). Numerous similar proteins from *Ralstonia* or *Pseudomonas* strains are involved in activation of chlorinated compounds feeding into the catechol branch of the β-ketoadipate pathway (s. Table 1). Two LysR-type regulators are involved in gene regulation of this pathway in *Acinetobacter* sp. strain ADP1. BenM is required for benzoate degradation (Collier *et al.*, 1998) and responds to *cic,cis*-muconate and benzoate. It is active in regulating not only expression of *ben* genes for conversion of benzoate into catechol, but also several *cat* genes. CatM in concert with *cic,cis*-muconate activates genes encoding the catechol branch of the β-ketoadipate pathway (Romero-Arroyo *et al.*, 1995). Thus, there exists some overlap in this regulatory system in *Acinetobacter* concerning both, effector and promoter specificity. One of many pathways feeding into the central reactions of the pathway, the conversion of alkyl salicylates into catechol by the *sal* encoded gene products is controlled by LysR-type regulator SalR (Jones *et al.*, 2000). SalR has also been described and purified from *P. putida* S-1 (Sato *et al.*, 2001). PcaQ from *Agrobacterium tumefaciens*, a LysR-type regulator was found to control expression of genes *pcaDCHGB* for conversion of protocatechuate into β-ketoadipate in concert with the two pathway metabolites β-carboxy-*cic,cis*-muconate and γ-carboxymuconolactone as effectors (Parke, 1996a). PcaQ seems to be abundant among *Rhizobium* species (Parke, 1996b). *PcaQ*-like genes were also identified on the DNA sequence level in several strains of the marine *Roseobacter* lineage of the α-proteobacteria clustered with *pca* genes (Buchan *et al.*, 2000). So far, LysR-type regulators have almost exclusively been found associated with the β-ketoadipate pathway. Most representatives are involved in the catechol branch, and a few examples control expression of the protocatechuate branch. The only exception is *E. coli* K12 HcaR, which regulates expression of enzymes for the initial catabolic steps of 3-phenylpropionic acid (Diaz *et al.*, 1998). Further metabolism of the aromatic intermediate is accomplished by the *mhp*-encoded *meta*-fission pathway controlled by an IclR family member.

Regulators of the IclR Group

This group is probably the most recently recognized family among proteins governing gene expression of catabolic pathways for aromatic compounds with a fast growing number of representatives. The *E. coli* IclR protein is the prototype of this family. It negatively controls expression of the enzymes forming the glyoxylate shunt (Nègre *et al.*, 1991), whereas all regulators within the so-called PobR subfamily are activators. The first regulator that was identified as a member of the new protein family was PobR from *Acinetobacter* sp. strain ADP1 (DiMarco *et al.*, 1993). PobR controls the expression of the *pobA* gene encoding the hydroxylase for conversion of *p*-hydroxybenzoate into protocatechuate, which then is degraded by *ortho*-cleavage through the β-ketoadipate pathway. A preliminary domain structure of PobR was deduced from a mutation analysis of the protein. The study revealed DNA binding function in the region around the HTH motif in the N-terminal part of PobR and residues important for inducer specificity in the central part (Kok *et al.*, 1998). All genes necessary for protocatechuate degradation into Krebs cycle intermediates are controlled by another IclR-type regulator, PcaU (Gerischer *et al.*, 1998). In addition, PcaU is suggested to be in control of *qui* gene expression. The latter genes are located directly downstream of the *pca* genes and convert quinate into protocatechuate. At elevated levels of the effector protocatechuate PcaU leads to activation of *pca* gene expression. In contrast, without addition of the effector it has a repressing effect at the structural gene promoter (Trautwein and Gerischer, 2001). This dual functionality at the same promoter is probably based on interactions between PcaU and RNA polymerase other than physical hindrance of polymerase binding. PcaU covers nucleotides –48 to –92 with respect to the transcription start site of the *pca* gene cluster (unpublished observation, R. Popp and U. Gerischer). This should be far enough away from the –10/–35 binding site for RNA polymerase to prevent overlap of binding sites. The 45 bp covered by PcaU in the intergenic region between the *pca* gene

cluster and the *pcaU* gene contain three perfect 10-bp repetitions, two of which form an inverted repeat and the third one a direct repeat. The two external repeats of this unusual regulator binding site, which is partly conserved in the PobR binding site, can be deleted without loss of specific PcaU binding, but the affinity of PcaU to these shortened sites decreases considerably (unpublished observation, R. Popp and U. Gerischer). The wild type affinity of the regulator to the intergenic binding site is important for induction at the structural gene promoter. This is concluded from the description of spontaneous mutant strains with altered or deleted external repeats. The growth phenotype of such strains on protocatechuate is strongly disturbed (D'Argenio *et al.*, 2001). Despite the high similarity between the PobR and PcaU proteins (50% identical residues), the similarity between the effector molecules (protocatechuate differs from *p*-hydroxybenzoate by one additional hydroxyl group), and between the binding sites (18 identical residues out of 21 residues forming the palindrome), there is no effector- or regulator-dependent cross regulation. PcaR from *P. putida* has the equivalent function to PcaU; the two proteins are 31% identical (Romero-Steiner *et al.*, 1994). The two proteins differ in the nature of the effector (PcaR is activated by β-ketoadipate, which is structurally unrelated to the aromatic protocatechuate) and of the binding site. The latter is well conserved within the palindrome, but its location completely covers the −10 region (of the *pcaR* promoter), or both, the −10 and −35 region of the *pcaIJ* promoter (Guo and Houghton, 1999). This suggests different molecular mechanisms for the activity of these two proteins. Other IclR-type regulators controlling *pca* gene expression were described in *Agrobacterium tumefaciens* and even in the evolutionary remote gram-positive *Rhodococcus opacus* (Table 1). The latter organism contains a second IclR-type protein. CatR is exceptional in that it controls the *cat* genes encoding enzymes for the catechol branch of the β-ketoadipate pathway. In all other known cases this branch is under control of LysR-type regulators (Eulberg and Schlömann, 1998). Based on DNA sequence data, HppR from *Rhodococcus globerulus* was identified as another IclR regulator from a gram-positive bacterium likely to be involved in regulation of 3-(3-hydroxyphenyl)propionic acid (Barnes *et al.*, 1997). The same pathway also exists in *E. coli* K12 enabling it to metabolize this aromatic carbon source. IclR-type regulator MhpR was shown to control the expression of the pathway (Ferrandez *et al.*, 1997).

Regulatory Protein Groups with Low Abundance in Regulation of Aromatic Compound Degradation

Three regulators have been identified as belonging into the MarR family of bacterial regulatory proteins. MarR negatively regulates the expression of the antibiotic resistance genes *marAB* with one effector being the aromatic compound 2-hydroxybenzoate (Martin and Rosner, 1995). BadR was identified as a member of this family. It positively regulates the *badDEFG* gene expression encoding benzoyl-coenzyme A reductase. This enzyme initiates the anaerobic breakdown in *Rhodopseudomonas palustris* (Egland and Harwood, 1999). It is likely to respond to benzoyl-CoA and is the first identified regulator of this family concerned with catabolic reactions for aromatic compounds. Besides, BadR acts as an activator rather than as a repressor. Another member of the MarR family is the *E. coli* C HpcR repressor. This protein controls the expression of genes for homoprotocatechuate degradation which proceeds via a *meta*-cleavage pathway similar to the one encoded on the TOL plasmid (Roper *et al.*, 1993). *E. coli* K12 is devoid of this gene cluster. Another *E. coli* aromatic degradation pathway enabling *E. coli* W to grow on 4-hydroxyphenylacetate was found to include two potential regulator genes associated with the respective gene clusters. HpaA has been mentioned earlier as an AraC/XylS group member, *hpaR* has coding capacity for a protein similar to other MarR group members (Prieto *et al.*, 1996).

A few examples of regulators are known, that are two-component systems. One example is StyS/StyR from *Pseudomonas* sp. strain Y2 activating the expression for *styABCD*, genes for the conversion of styrene into phenylacetate (Velasco *et al.*, 1998). The StyR DNA binding domain shows similarity to that of class 3 response regulators which appear to interact with RNA polymerases containing various sigma factors (Reizer and Saier, 1997). A StyR binding site appears to be centered at −41 upstream of the *styA* transcriptional start site, which would suggest a different regulatory mechanism from TodT because the latter binds around −105. TodS and TodT were described to govern the expression of the toluene-degrading route in *P. putida* F1 (Lau *et al.*, 1997). The TodS sensor protein is unique in that its N-terminus displays a bZIP motif consisting of a DNA binding region and a leucine zipper region. This motif is common among eukaryotic transcriptional factors and mediates dimerization. Experiments with synthetic peptides proofed this function also for TodS. Another exceptional quality of TodS is that it contains a duplicated histidine kinase domain. TodS is a cytoplasmic protein, and the effector is probably entering the cell with the help of the outer-membrane protein TodX. This creates a much more specific response as compared to other regulators, for example DmpR or XylR. TutB/TutC from *Thauera aromatica* strain T1 form a two-component system and are suggested to be involved in the regulation of toluene utilization in this organism (Coschigano and Young, 1997).

In a few occasions members of the GntR family are involved in degradative pathways for aromatic compounds (Haydon and Guest, 1991). VanR is a repressor of *vanA,B* gene expression, mediating conversion of vanillate into protocatechuate by demethylation in *Acinetobacter* sp. strain ADP1 (Morawski *et al.*, 2000). Genes similar to *vanR* have also been observed in *Pseudomonas* strains (Priefert *et al.*, 1997). Another GntR-type repressor has been described in two strains of *Comamonas testosteroni* and is involved in the regulation of phenol hydroxylase (Arai *et al.*, 1999; Teramoto *et al.*, 2001). In these organisms, a DmpR homologue is necessary for the induction of the enzyme, but unless the proteins PhcS

or AphS respectively are present, expression is more or less constitutive. Here, the DmpR-like protein is necessary to enable transcription and the GntR-type protein brings about the specificity of expression in response to phenol. Low levels of similarity to the GntR family are observed in two more cases. PaaX controls expression of the *paa* genes in *E. coli* K12 for phenylacetic acid degradation (Ferrandez *et al.*, 2000). It is unique in that its effector is an aryl-CoA compound (phenylacetic acid CoA). CymR was shown to repress *p*-cymene conversion to *p*-cumate in *P. putida* F1 (Eaton, 1997). Thus, all the GntR group members show the activity mode of a repressor which is the exception in the group of regulators discussed in this communication.

Involvement of proteins belonging to the FNR-CRP superfamily in regulation of aromatic compound degradative pathways has been demonstrated in the denitrifying bacterium *Rhodopseudomonas palustris*. Two members of this family are involved in regulation of anaerobic *p*-hydroxybenzoate conversion to benzoyl-CoA (Dispensa *et al.*, 1992; Egland and Harwood, 2000). HbaR was required for *p*-hydroxybenzoate-specific induction of expression of the first enzyme, but this response was not depending on the oxygen level. Several lines of evidence indicate that the specificity of the response towards anaerobic conditions is brought about by an FNR homologue, called AadR, which activates expression of *hbaR* only under anaerobic conditions.

Superimposed Levels of Regulation

Besides the different ways of specific gene regulation, there are superimposed mechanisms of regulation. Some of these, for example in *E. coli*, are well understood whereas others are only in the beginning stages of being described. These higher levels of regulation are i) mechanisms that create a hierarchy between individual catabolic pathways in the simultaneous presence of the respective substrates, or ii) mechanisms causing preferred carbon sources to be degraded preferentially by repressing larger sets of specialized degradation pathways (generally referred to as carbon catabolite repression (Stülke and Hillen, 1999)), or iii) mechanisms involving (p)ppGpp-mediated regulation or growth phase-dependent regulation. Here, only a few examples can be named among the many descriptions of the three different phenomenons. The two branches of the β-ketoadipate pathway are not used simultaneously when substrates of either branch (for example benzoate and *p*-hydroxybenzoate) are present. Benzoate is used first and during this time, expression of genes for the degradation of the other substrate is repressed. This was observed in *P. putida* and in *Acinetobacter* sp. strain ADP1 (Gaines III *et al.*, 1996; Nichols and Harwood, 1995). In *P. putida* the XylS homologue BenR was shown to be involved indirectly in this repression, but for the most part the underlying mechanism is not known. In a large number of cases repression of genes for degradation of aromatic compounds by preferred carbon sources has been observed (Collier *et al.*, 1996). In *E. coli* this hierarchy is brought about by the activity of the well-described catabolite activator protein (Stülke and Hillen, 1999). In organisms closely related to *Pseudomonas* or *Acinetobacter*, these preferred carbon sources are mostly organic acids, for example succinate. The underlying mechanism is most likely different from the systems described in enteric bacteria or in gram-positive species (reviewed in Research in Microbiology, 1996, no. 6–7). A few clues revealed aspects of such mechanisms like the involvement of the specific regulator (Müller *et al.*, 1996), the direct binding of a preferred carbon source to the specific regulator (McFall *et al.*, 1997), or the involvement of the PtsN protein (Cases *et al.*, 1999; Du *et al.*, 1996). In *P. aeruginosa* the Crc protein has been shown to be involved in catabolite repression at multiple operons, but again the activity of this protein is unknown (MacGregor *et al.*, 1996). Growth phase-dependant gene expression has been observed (Sze *et al.*, 1996) and could be linked with regulatory effects brought about by the alarmone (p)ppGpp (Carmona *et al.*, 2000; Sze and Shingler, 1999).

Conclusions

Comparing proteins and mechanism involved in regulation of aromatic compound degradation means looking at a small portion of all regulatory proteins described so far. In this review it becomes obvious that within this subgroup preferences for members of certain regulator families exist. The LysR, AraC/XylS, XylR/NtrC and the IclR families are groups with the most representatives. In general there are also correlations between certain degradative pathways and certain regulator groups. For example the *ortho*-cleavage through the β-ketoadipate pathway is controlled by regulators from the LysR and the IclR families. Between the two branches starting from catechol or protocatechuate there is also preference in that LysR-type regulators control the catechol branch and IclR-type regulators control the protocatechuate branch. Regulators of pathways for degradation of aromatic compounds have most intensely been studied in γ-proteobacteria but the use of IclR-type regulators for the regulation of protocatechuate degradation is conserved even in the gram-positive *Rhodococcus*. Some examples point to the opposite scenario which is that genes for metabolic pathways and their regulators do not share a common history. For example regulation of *p*-hydroxybenzoate hydroxylase is in several bacteria accomplished by AraC-type proteins whereas *Acinetobacter* sp. strain ADP1 employs an IclR family member (in all cases named PobR). Pathways for anaerobic degradation of aromatic compounds (Harwood and Gibson, 1997) are different from the aerobic routes. In light of the overall conservation of cooperation between certain regulator groups with certain pathways it seems plausible that regulators for anaerobic pathways are related to other protein

families. Different ways of controlling gene expression on higher levels integrating the physiological status of the cell into the level of promoter activity exist. Molecular mechanisms for these global systems are only in the beginning stages to be understood. Besides the scientific interest in new systems of global gene regulation, there is also broad interest from the biotechnological point of view since these pathways play an important role in bioremediation (Diaz and Prieto, 2000) or in the use of regulatory proteins as biosensors (Applegate *et al.*, 1998).

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