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The TetR Family of Transcriptional Repressors

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INTRODUCTION

Bacteria in the environment are exposed to variations in temperature and nutrient and water availability and the presence of toxic molecules that originate from their abiotic and biotic surroundings (including deleterious molecules that originate from their own metabolism). These changes can make their living conditions far from optimal. Survival in this unstable environment requires a wide range of rapid, adaptive responses which are triggered by regulatory proteins. These regulators respond to specific environmental and cellular signals that modulate transcription, translation, or some other event in gene expression, so that the physiological responses are modified appropriately (32, 52, 64, 104, 107, 145, 244, 311, 312, 326, 330, 379, 397, 409, 427).

In most cases, the adaptive responses are mediated by transcriptional regulators. Most microbial regulators involved in transcriptional control are two-domain proteins with a signal-receiving domain and a DNA-binding domain which transduces the signal (1, 18, 145, 152, 170, 207, 271, 292–294, 298, 303, 345, 369, 428, 431) (Table 1). In other cases, the sensing of signals that trigger a transcriptional process involves two proteins, as in two-component regulatory systems such as CzcR/CzcS; DcuS/DcuR; NifL/NifA; NtrB/NtrC; PhoP/PhoQ; and TodS/TodT (75, 139, 200, 206, 233, 234, 257, 307, 309, 316, 409, 423). One protein is usually a membrane-linked kinase that, upon sensing the appropriate signal, phosphorylates a DNA-binding protein that mediates transcription from its cognate promoter. Structural analyses have revealed that the helix-turn-helix (HTH) signature is the most recurrent DNA-binding motif in prokaryotic transcriptional factors, since almost 95% of all transcriptional factors described in prokaryotes use the HTH motif to bind their target DNA sequences (12, 19, 27, 41, 43, 104, 135, 136, 302, 335, 343).

Prokaryotic transcriptional regulators are classified in fami-

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TABLE 1. Prokaryotic regulator families

Family	Action	Some regulated functions	DBD motif	Position	Reference(s)
LysR	Activator/repressor	Carbon and nitrogen metabolism	HTH	N-terminal	145, 342
AraC/XylS	Activator	Carbon metabolism, stress response and pathogenesis	HTH	C-terminal	109, 394
TetR	Repressor	Biosynthesis of antibiotics, efflux pumps, osmotic stress, etc.	HTH	C-terminal	9, 10, 11
LuxR	Activator	Quorum sensing, biosynthesis and metabolism, etc.	HTH	C-terminal	106, 298, 317
LacI	Repressor	Carbon source utilization	HTH	N-terminal	54, 420
ArsR	Repressor	Metal resistance	HTH	Central	49, 432
IcIR	Repressor/activator	Carbon metabolism, efflux pumps	HTH	N-terminal	265, 319, 321, 378
MerR	Repressor	Resistance and detoxification	HTH	N-terminal	144, 377
AsnC	Activator/repressor	Amino acid biosynthesis	HTH	N-terminal	103
MarR	Activator/repressor	Multiple antibiotic resistance	HTH	Central	4, 13, 352, 376
NtrC (EBP)	Activator	Nitrogen assimilation, aromatic amino acid synthesis, flagella, catabolic pathways, phage response, etc.	HTH	C-terminal	200, 257
OmpR	Activator	Heavy metal and virulence (response regulator of a two-component system)	Winged helix	C-terminal	237
DeoR	Repressor	Sugar metabolism	HTH	N-terminal	311, 405, 450
Cold shock	Activator	Low-temperature resistance	RNA binding domain (CSD)	Variable	42, 205, 344
GntR	Repressor	General metabolism	HTH	N-terminal	138, 318, 324
Crp	Activator/repressor	Global responses, catabolite repression and anaerobiosis	HTH	C-terminal	54, 110, 244

lies on the basis of sequence similarity and structural and functional criteria (49, 84, 106, 108, 120, 121, 138, 145, 146, 237, 274, 308, 313, 324, 342, 370, 377). Table 1 lists the most important families of microbial transcriptional regulators, the type of DNA binding motifs they exhibit, whether the members of the family are preferentially repressors or activators, and whether they show a dual action.

This review focuses on the TetR family, a family of transcriptional regulators that is well represented and widely distributed among bacteria with an HTH DNA-binding motif (210, 211, 246, 288).

Members of the TetR family of repressors are identified by a profile (see below) which can be easily used to recognize TetR family members in SWISS-PROT and TrEMBL and in all available proteins from prokaryotic genome sequences. After compiling data from protein and nucleic acid databases, the TetR family of regulators was found to include 2,353 nonredundant sequences (as of December 2004). The specific function regulated by members of the TetR family is known for only about 85 members (Table 2). These proteins control genes whose products are involved in multidrug resistance, enzymes implicated in different catabolic pathways, biosynthesis of antibiotics, osmotic stress, and pathogenicity of gram-negative and gram-positive bacteria (Table 2). The most relevant information on these proteins is collected in a database available at http://www.bactregulators.org (235). The database also supplies information for each member of the family, including identifiers, names, sequences, source, function, COG (clusters orthologous groups), position and orientation of the corresponding gene in the genome, and, when available, three-dimensional structures.

DEFINING THE TetR FAMILY
TetR Family Profile

The TetR family is named after the member of this group that has been most completely characterized genetically and biochemically, the TetR protein (141, 148, 150, 168, 288, 395).

This protein controls the expression of the *tet* genes, whose products confer resistance to tetracycline (150, 183, 209, 210, 337, 434, 435). Members of the TetR family exhibit a high degree of sequence similarity at the DNA binding domain (see below). Interpro (258) assigns proteins to the TetR family based on PROSITE signature PS01081 (364), PRINTS motif PR00455 (15, 16), and Pfam Hidden Markov Model (HMM) profile PF00440 (26, 27). To establish a single criterion defining the TetR family, we decided to develop a conventional profile, because conventional profiles are easy to manage and their sensitivity is equivalent to that of HMM profiles.

To develop the TetR family profile, we first selected a set of 120 sequences as belonging to the TetR family based on two criteria: a positive score for PROSITE signature PS01081, and a high score for PF00440 HMM. The 120 sequences were clustered into 42 groups using BLAST, and a representative sequence was selected and aligned for each cluster using CLUSTAL (http://clustalw.genome.ad.jp/). This revealed that the most conserved region corresponded to the HTH domain described in the TetR and QacR crystals (120, 150, 287, 288, 289, 349, 350, 351). The initial HTH motif was progressively extended until the global score of the multialignment diminished. Figure 1 shows the final alignment of the sequences. This conserved stretch corresponded in TetR and QacR crystals to the almost complete α-helix 1, the HTH domain formed by α-helices 2 and 3, and five residues of α-helix 4 that connect the DNA-interacting region with the core of the protein (see Fig. 2 for the three-dimensional structure of TetR).

The final alignment shown in Fig. 1 was used as a seed for the construction of a conventional profile to detect TetR family members. The TetR profile was built using the pfmake program available at the Swiss Institute of Bioinformatics (http://npsa-pbil.ibcp.fr/cgi-bin/npsa__automat.pl?page=/NPSA/npsa__pfmake.html) (45, 46). The TetR profile was confronted against the 660,992 bacterial and archaeal proteins in the SWISS-PROT and TrEMBL databases (released December

TABLE 2. Specific functions regulated by members of the TetR family of repressors

No.	SPTR[a]	Name	Organism	Function	G[b]	Reference(s)
1	P34000	AcrR	*Escherichia coli*	Represses the expression of the *acrAB* operon which confers multidrug resistance and probably also controls the gene *micF*	1	102, 164, 223, 224, 314, 325, 425
2	Q53901	ActII	*Streptomyces coelicolor*	Located in the *act* cluster, which contains regulatory and antibiotic export genes	1	50, 96
3	Q9F8V9	AmeR	*Agrobacterium tumefaciens*	Negatively regulates the *ameABC* operon, which encodes proteins similar to nodulation-cell division (RND)-type efflux systems	1	301
4	Q9RG61	AmrR	*Pseudomonas aeruginosa*	Probably regulates *amrAB* genes encoding an efflux system involved in aminoglycoside impermeability phenotype in *Pseudomonas aeruginosa*	1	423
5	Q9KJC4	ArpR	*Pseudomonas putida* S12	Seems to be a repressor for the expression of the *arpABC* operon; ArpABC in *Pseudomonas putida* S12 is involved only in multidrug resistance and not in tolerance towards organic solvents.	1	178
6	Q6VV70	BpeR	*Burkholderia pseudomallel*	Controls expression of the BpeAB-OprB efflux pump that extrudes gentamicin, streptomycin erythromycin, and acryflavine	1	53
7	P31676	EnvR	*E. coli* K-12	Regulates the *acrEF* efflux pump operon, which is relevant to multidrug resistance in *E. coli*. Its substrate specificity (antibiotics, basic dyes and detergents) is similar to that of AcrAB	1	186
8	P96222	EthR	*Mycobacterium tuberculosis*	Ethionamide resistance	1	28, 90, 111
9	P72185	HemR	*Propionibacterium freudenreichii*	Probably regulates *hemX*, which appears to be involved in heme transport	1	137
10	Q93QZ7	HydR	Tn*5398* from *Clostridium difficile*	Involved in erythromycin resistance	1	93
11	O68442	IfeR	*Agrobacterium tumefaciens* 1D1609	Seems to be a repressor that controls the expression of the putative *ifeABR* isoflavonoid efflux system	1	296
12	Q9ZGB7	LanK	*Streptomyces cyanogenus*	Probably a landomycin A resistance regulator	1	315, 424
13		LfrR	*Mycobacterium segmatis*	Control of the *lfrA* gene whose end product confers resistance to fluoroquinolones, ethidium bromide, and acryflavine	1	212
14	O34619	LmrA	*Bacillus subtilis*	Probable repressor of the lincomycin-resistance operon	1	197, 198, 260
15	P39897	MtrR	*Neisseria gonorrhoeae*	A transcriptional repressor that regulates transcription of the *mtrCDE* genes, which encode a multidrug efflux pump; MtrR acts directly or indirectly as a positive regulator of *farAB* gene expression	1	72, 130, 131, 220, 221, 297, 333, 334, 339, 447, 448
16	Q9F0Y2	Pip	*Streptomyces coelicolor*	Pristinamycin I-induced regulator that controls mutidrug resistance genes	1	99
17	Q9F147	PqrA	*Streptomyces coelicolor*	Probably the repressor of *pqrB*, which encodes an efflux pump conferring resistance to paraquat	1	61
18	P23217	QacR	*Staphylococcus aureus*	Regulates the QacA multidrug efflux pump	1	119, 120, 249, 299, 300, 332, 350, 351, 390
19	O52558	RifQ	*Amycolatopsis mediterranei*	Located in the rifamycin biosynthetic gene cluster and probably related to the adjacent gene that encodes a rifamycin efflux protein	1	17
20	Q9KIH5	RmrR	*Rhizobium etli* plasmid B	Probably regulates the operon *rmrAB* related to a multidrug efflux pump involved in sensitivity to phytoalexins, flavonoids, and salicylic acids	1	113
21	Q9AMH9	SimReg 2	*Streptomyces antibioticus*	Included in the *Streptomyces antibioticus* simocyclinone biosynthetic gene cluster; probably regulates the putative export protein SimEX	1	396
22	Q8KLP4	SmeT	*Stenotrophomonas maltophilia*	A repressor of the *Stenotrophomonas maltophilia* multidrug efflux pump SmeDEF	1	340, 452
23	Q9R9T9	SrpR	*Pseudomonas putida*	Probable regulator of the solvent resistance pump SrpABC of strain S12	1	163, 179, 180, 422
24	P39885	TcmR	*Streptomyces glaucescens*	A regulator of the tetracenomycin C resistance repressing the gene *tcmA*, which encodes an export pump	1	126, 127
25	P09164	TetR	*Escherichia coli*	Controls the expression of tetracycline resistance mediated by the gene *tetA*, which encodes an efflux pump that acts as an antiporter by coupling the export of [MgTetracycline]$^+$ out of the cell with the uptake of protons	1	29, 30, 36, 38, 39, 142, 143, 148, 149, 150, 183, 189, 259, 286, 287, 288, 289, 393, 402, 430
26	Q9AIU0	TtgR	*Pseudomonas putida*	Regulates the TtgABC efflux pump mediating organic solvent tolerance and resistance to ampicillin, tetracycline, chloramphenicol, and nalidixic acid	1	81, 391
27	Q93PU7	TtgW	*Pseudomonas putida*	*ttgW* is a pseudogene	1	327, 329
28	Q9RP98	UrdK	*Streptomyces fradiae* Tu2717	Probably regulates an urdamycinA efflux pump	1	94
29	Q9AJL5	VarR	*Streptomyces virginiae*	Regulates transcription of *varS*, the virginiamycin S-specific transporter in a virginiamycin S-dependent manner	1	263
30	P96676	YdeS	*Bacillus subtilis*	Similar to a regulator of antibiotic transport complexes in *Streptomyces hygroscopicus*	1	34
31	Q54189	ArpA	*Streptomyces griseus*	Represses the expression of *adpA*; AdpA activates the expression of *strR*, and the StrR protein activates the expression of streptomycin biosynthetic genes. ArpA also controls morphogenesis	2, 5, 8	157, 278, 282, 283, 285, 375, 412, 438

Continued on facing page

TABLE 2—*Continued*

No.	SPTR[a]	Name	Organism	Function	G[b]	Reference(s)
32	Q93M20	Aur1B	*Streptomyces aureofaciens*	Included in the *Streptomyces aureofaciens* auricin polyketide biosynthesis gene cluster	2	275
33	Q9LBV6	BarA	*Streptomyces virginiae*	Probably involved in regulation of virginiamycin biosynthesis	2	175, 262
34	Q8KNI9	CalR1	*Micromonospora echinospora*	Included in the calicheamicin gene cluster	2	2
35	O66129	CprB	*Streptomyces coelicolor*	CprB is involved in the control of actinorhodin and undecylprodigiosin biosynthesis and morphogenesis	2	264, 284
36	O24741	FarA	*Streptomyces lavendulae* FRI-5	IM-2-specific receptor; plays an important role in the regulation of secondary metabolism and the biosynthesis of the antibiotics showdomycin and minimycin in *Streptomyces lavendulae*; FarA acts as a negative transcriptional regulator for the biosynthesis of nucleoside antibiotics and blue pigment, switching on their expression in the presence of IM-2; also acts as a positive transcriptional regulator for the biosynthesis of D-cycloserine, switching off its expression in the presence of IM-2	2	184, 185, 413
37	Q939Q2	JadR*	*Streptomyces venezuelae*	Included in the cluster for the biosynthesis of the dideoxysugar component of jadomycin B	2	416
38	Q56153	JadR2	*Streptomyces venezuelae*	Represses the biosynthesis of jadomycin B and seems to control cellular pigmentation	2	442, 443
39	Q9ZN97	MphB	*Escherichia coli* plasmid pTZ3721	Repressor of antibiotic biosynthesis	2	172, 272
40	Q9XDF0	NonG	*Streptomyces griseus* sbsp. *griseus*	Probably related to nonactin biosynthesis	2	414
41	Q9RF02	PhlF	*Pseudomonas fluorescens*	A repressor of the *phlABCD* operon responsible for the biosynthesis of the antifungal 2,4-diacetylphloroglucinol (PHL)	2	346
42	Q9ZHP8	TylQ	*Streptomyces fradiae*	Butyrolactone receptor TylQ is a potential regulator of production of the macrolide antibiotic tylosin	2, 8	371
43	Q8VQC6	VanT	*Vibrio anguillarum*	Positively regulates serine metalloprotease, pigment and biofilm production	2, 5	71
44	Q9RPK9	TarA	*Streptomyces tendae*	Hypothetical receptor of gamma-butyrolactone, which regulates nikkomycin synthesis	2, 8	86
45	Q9XCC7	TylP	*Streptomyces fradiae*	Regulates tylosin production and morphological differentiation, and is probably a gamma-butyrolactone receptor	2, 5, 8	25, 371, 372
46	Q59213	BM1P1	*Bacillus megaterium*	Probably acts as positive regulatory protein involved in the expression of the P450BM-1 gene by interfering with the binding of the repressor protein, Bm3R1, to the regulatory regions of P450BM-1	3	358, 361, 362
47	O68276	Bm1P1	*Bacillus megaterium* ATCC 14581	Negatively affects basal-level expression of P450BM-1, a barbiturate-inducible P450 monooxygenase; cytochromes P450BM-3 and P450BM-1 catalyze the hydroxylation of fatty acids	3	140, 214, 215, 295, 356, 358, 359, 362
48	P43506	Bm3R1	*Bacillus megaterium*	A transcriptional repressor involved in the regulation of barbiturate-inducible proteins in *Bacillus megaterium*	3	87, 88, 89, 140, 213, 214, 295, 356, 357, 358, 359, 361, 362
49	Q9AJ68	ButR	*Streptomyces cinnamonensis*	Putative transcriptional repressor of crotonyl-CoA reductase	3	218, 219
50	Q93TU7	CampR	*Rhodococcus* sp. NCIMB 9784	Probably regulates 6-oxocamphor hydrolase	3	123
51	Q51597	CamR	*Pseudomonas putida* plasmid CAM	A negative regulator of the cytochrome P-450cam hydroxylase operon	3	9, 10, 105
52	O33453	CymR	*Pseudomonas putida*	A repressor which controls expression of both the *cym* and *cmt* operons and is inducible by *p*-cumate but not *p*-cymene	3	62, 82, 83, 279
53	Q9RAJ1	DhaR	*Mycobacterium* sp. GP1	Appears to function as a repressor of *dhaA* expression, *dhaA* is an haloalkane dehalogenase gene included in the 1-clorobutane catabolic gene cluster	3	306
54	Q9RA03	KstR	*Rhodococcus erythropolis* strain SQ1	A repressor of *kstD* expression that encodes a 3-ketosteroid Δ-dehydrogenase protein involved in the degradation of steroid intermediates in phytosterol degradation	3	404
55	Q8VV87	LexA-like	*Terrabacter* sp. strain DBF63	Probably involved in degradation of dibenzofuran	3	171
56		AcnR	*Corynebacterium glutamicum*	Repressor of the *acn* gene encoding aconitrase and controlling the tricarboxylic acid cycle	3	195
57	Q9FA56	PaaR	*Azoarcus evanssi*	Probably regulates the *paa* genes, which are responsible for the aerobic phenylacetic acid catabolic pathway	3	256
58	Q9XDW2	PsbI	*Rhodopseudomonas palustris*	Included in the cluster of genes participating in aerobic biodegradation of *p*-cumate	3	310

TABLE 2—*Continued*

No.	SPTR[a]	Name	Organism	Function	G[b]	Reference(s)
59	O85706	ThlR	*Clostridium acetobutylicum* DSM 792	Possibly acts as a transcriptional repressor of the *thlRBC* operon, which is involved in the biosynthesis of thiolase	3	429
60	Q59431	UidR	*E. coli*	A repressor of the *uidRABC* (*gusRABC*) operon that comprises a beta-D-glucuronidase (*uidA*), a glucuronide permease (*uidB*) and a membrane-associated protein (*uidC*)	3	40
61	P22645	YDH1	*Xanthobacter autotrophicus*	Probably regulates the *dhlA* gene involved in 1,2-dichloroethane degradation	3	162
62	P17446	BetI	*Escherichia coli*	A choline-sensing repressor of the *bet* regulon involved in osmotic stress	4	8, 201, 202, 331
63	Q8NLK1	McbR	*Corynebacterium glutamicum*	In absence of L-methionine, represses the expression of six key enzymes for the biosynthesis of the sulfur-containing amino acids L-cysteine and L-methionine including sulfonate utilization and sulfite reduction	4	322
64	Q9EVJ6	MphR	*Escherichia coli*	Represses the *mph(A)-mrx-mphR(A)* operon in the absence of erythromycin; erytromycin induces the synthesis of macrolide 2′-phosphotransferase I [Mph(A)], which inactivates erythromycin	4	273
65	Q9F9Z7	PhaD	*Pseudomonas oleovorans*	Biosynthesis of medium-chain-length (MCL) poly-3-hydroxyalkanoates (PHAs) as intracellular storage material	4	188, 451
66	Q9ZF45	Q9ZF45	*Lactococcus lactis*	Regulates the operon *purDEK*, which encodes enzymes in the de novo pathway of purine nucleotides	4	269
67	P06969	TtK	*Escherichia coli*	Co-transcribed with the *dut* (deoxyuridine triphosphatase) gene	4	85, 415
68	P32398	Yhgd or YixD	*Bacillus subtilis*	Probably related to protoheme IX biosynthesis	4	132
69	Q9F6W0	CasR	*Rhizobium etlli*	A repressor of the *casA* gene, which encodes the calmodulin-like protein calsymin involved in bacteroid development during symbiosis and in symbiotic nitrogen fixation	5	433
70	Q9RQQ0	IcaR	*Staphylococcus aureus*	A repressor of the operon *ica* which is responsible for an intercellular polysaccharide compound that acts as the slime in biofilm formation	5	70, 163
71	Q8GLC6	IcaR	*Staphylococcus epidermidis*	A repressor of the operon *ica*, which is reponsible for an intracellular polysaccharide compound that acts as the slime in biofilm formation	5	60, 66, 67, 190, 456
72	Q8KX64	LitR	*Vibrio fischeri*	Important for the normal induction of luminescence, plays a positive role in modulating the ability to colonize juvenile squid, and may control the opacity/translucent phenotype of the colony	5, 8	97
73	P21308	LuxR	*Vibrio harveyi*	Required for expression of the *luxCDABEGH* (luciferase) operon, responsible for bacterial luminescence	5	23, 24, 51, 57, 167, 232, 240, 250, 251, 253, 254, 255, 355, 365, 380, 381, 382
74	Q9ANS7	LuxT	*Vibrio harveyi*	Activates the expression of LuxO, the phosphorelay protein that regulates luminescence in *Vibrio harveyi*	5	216
75	O50285	OpaR	*Vibrio parahaemolyticus*	A transcriptional regulator that controls the opaque morphology in *Vibrio parahaemolyticus* colonies	5	240, 355
76	Q9XDV7	Orf2	*Streptomyces griseus*	Probably related to carbon-source-dependent differentiation in *Streptomyces griseus*	5	398
77	Q9L8G8	SmcR	*Vibrio vulnificus*	Appears to play an important role in starvation adaptation and in the regulation of many growth phase-regulated genes, including some virulence factors (protease, hemolysin); ScmR represses motility, fimbria production, and biofilm production	5 6	63, 242, 243, 355
78	O30343	HapR	*Vibrio cholerae*	A transcriptional regulator with a central role in control of the virulence of *Vibrio cholerae*, in a cell density-dependent way	6	165, 194, 196, 240, 455
79	Q8KU49	Ef0113	*Enterococcus faecalis*	Located in a pathogenicity island in vancomycin-resistant *Enterococcus faecalis*	6	354
80	Q63B57	HlyIIR	*Bacillus cereus*	Regulates expression of *hly*II whose gene product has haemolytic activity	6	47
80	O24739	BarB	*Streptomyces virginiae*	Regulates virginiamycin biosynthesis	7	182
81	O86852	ScbR	*Streptomyces coelicolor*	Acts as the cytoplasmic receptor that specifically binds SCB1 gamma-butarylactone and negatively regulates transcription of the *scbA* gene, responsible for gamma-butyrolactone SCB1 synthesis	2, 7, 8	3, 385, 386
82	Q9JN89	MmfR	*Streptomyces coelicolor* plasmid SCP1	Putative lactone-dependent transcriptional regulator	8	437
83	Q9S3L4	AmtR	*Corynebacterium glutamicum*	Regulator of nitrogen control	9	48,161
84	Q9EX90	PsrA	*Pseudomonas putida*	Involved in the regulatory cascade controlling *rpoS* gene regulation in response to cell density	9	192
85	P36656	YjdC	*Escherichia coli*	Probably involved in copper tolerance	10	101

[a] Swiss-Prot and TrEMBL accession number.

[b] 1, regulation of efflux pumps and transporters involved in antibiotic resistance and tolerance to toxic compounds; 2, regulation of antibiotic biosynthesis; 3, regulation of catabolic pathways; 4, biosynthesis of products important for bacteria (e.g., osmoprotectants, nucleotides, amino acids, PHAs, protoheme); 5, regulation of differentiation (sporulation, mycelium formation), colony phenotype, biofilm formation; 6, regulation of genes involved in virulence; 7, regulation of butyrolactone synthesis; 8, butyrolactone or autoinducer receptors; 9, global regulation; 10, other.
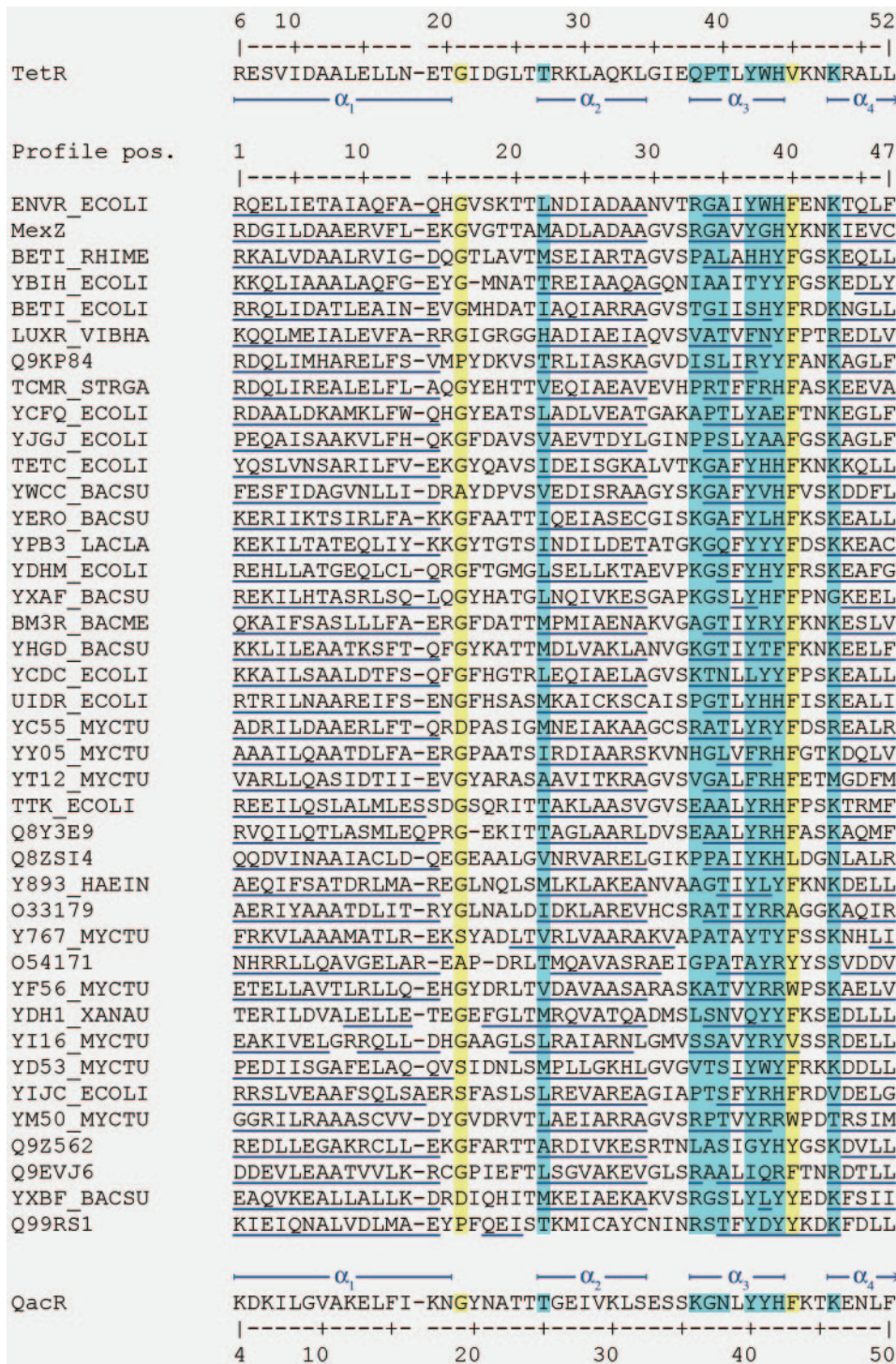
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                    6  10          20          30          40          52
                    |---+----+--- -+----+----+----+----+----+----+-|
TetR                RESVIDAALELLN-ETGIDGLTTRKLAQKLGIEQPTLYWHVKNKRALL
                        ————— α₁ —————           — α₂ —    — α₃ —  — α₄ →

Profile pos.        1          10          20          30          40    47
                    |---+----+--- -+----+----+----+----+----+----+-|
ENVR_ECOLI          RQELIETAIAQFA-QHGVSKTTLNDIADAANVTRGAIYWHFENKTQLF
MexZ                RDGILDAAERVFL-EKGVGTTAMADLADAAGVSRGAVYGHYKNKIEVC
BETI_RHIME          RKALVDAALRVIG-DQGTLAVTMSEIARTAGVSPALAHHYFGSKEQLL
YBIH_ECOLI          KKQLIAAALAQFG-EYG-MNATTREIAAQAGQNIAAITYYFGSKEDLY
BETI_ECOLI          RRQLIDATLEAIN-EVGMHDATIAQIARRAGVSTGIISHYFRDKNGLL
LUXR_VIBHA          KQQLMEIALEVFA-RRGIGRGGHADIAEIAQVSVATVFNYFPTREDLV
Q9KP84              RDQLIMHARELFS-VMPYDKVSTRLIASKAGVDISLIRYYFANKAGLF
TCMR_STRGA          RDQLIREALELFL-AQGYEHTTVEQIAEAVEVHPRTFFRHFASKEEVA
YCFQ_ECOLI          RDAALDKAMKLFW-QHGYEATSLADLVEATGAKAPTLYAEFTNKEGLF
YJGJ_ECOLI          PEQAISAAKVLFH-QKGFDAVSVAEVTDYLGINPPSLYAAFGSKAGLF
TETC_ECOLI          YQSLVNSARILFV-EKGYQAVSIDEISGKALVTKGAFYHHFKNKKQLL
YWCC_BACSU          FESFIDAGVNLLI-DRAYDPVSVEDISRAAGYSKGAFYVHFVSKDDFL
YERO_BACSU          KERIIKTSIRLFA-KKGFAATTIQEIASECGISKGAFYLHFKSKEALL
YPB3_LACLA          KEKILTATEQLIY-KKGYTGTSINDILDETATGKGQFYYYFDSKKEAC
YDHM_ECOLI          REHLLATGEQLCL-QRGFTGMGLSELLKTAEVPKGSFYHYFRSKEAFG
YXAF_BACSU          REKILHTASRLSQ-LQGYHATGLNQIVKESGAPKGSLYHFFPNGKEEL
BM3R_BACME          QKAIFSASLLLFA-ERGFDATTMPMIAENAKVGAGTIYRYFKNKESLV
YHGD_BACSU          KKLILEAATKSFT-QFGYKATTMDLVAKLANVGKGTIYTFFKNKEELF
YCDC_ECOLI          KKAILSAALDTFS-QFGFHGTRLEQIAELAGVSKTNLLYYFPSKEALL
UIDR_ECOLI          RTRILNAAREIFS-ENGFHSASMKAICKSCAISPGTLYHHFISKEALI
YC55_MYCTU          ADRILDAAERLFT-QRDPASIGMNEIAKAAGCSRATLYRYFDSREALR
YY05_MYCTU          AAAILQAATDLFA-ERGPAATSIRDIAARSKVNHGLVFRHFGTKDQLV
YT12_MYCTU          VARLLQASIDTII-EVGYARASAAVITKRAGVSVGALFRHFETMGDFM
TTK_ECOLI           REEILQSLALMLESSDGSQRITTAKLAASVGVSEAALYRHFPSKTRMF
Q8Y3E9              RVQILQTLASMLEQPRG-EKITTAGLAARLDVSEAALYRHFASKAQMF
Q8ZSI4              QQDVINAAIACLD-QEGEAALGVNRVARELGIKPPAIYKHLDGNLALR
Y893_HAEIN          AEQIFSATDRLMA-REGLNQLSMLKLAKEANVAAGTIYLYFKNKDELL
O33179              AERIYAAATDLIT-RYGLNALDIDKLAREVHCSRATIYRRAGGKAQIR
Y767_MYCTU          FRKVLAAAMATLR-EKSYADLTVRLVAARAKVAPATAYTYFSSKNHLI
O54171              NHRRLLQAVGELAR-EAP-DRLTMQAVASRAEIGPATAYRYYSSVDDV
YF56_MYCTU          ETELLAVTLRLLQ-EHGYDRLTVDAVAASARASKATVYRRWPSKAELV
YDH1_XANAU          TERILDVALELLE-TEGEFGLTMRQVATQADMSLSNVQYYFKSEDLLL
YI16_MYCTU          EAKIVELGRRQLL-DHGAAGLSLRAIARNLGMVSSAVYRYVSSRDELL
YD53_MYCTU          PEDIISGAFELAQ-QVSIDNLSMPLLGKHLGVGVTSIYWYFRKKDDLL
YIJC_ECOLI          RRSLVEAAFSQLSAERSFASLSLREVAREAGIAPTSFYRHFRDVDELG
YM50_MYCTU          GGRILRAAASCVV-DYGVDRVTLAEIARRAGVSRPTVYRRWPDTRSIM
Q9Z562              REDLLEGAKRCLL-EKGFARTTARDIVKESRTNLASIGYHYGSKDVLL
Q9EVJ6              DDEVLEAATVVLK-RCGPIEFTLSGVAKEVGLSRAALIQRFTNRDTLL
YXBF_BACSU          EAQVKEALLALLK-DRDIQHITMKEIAEKAKVSRGSLYLYYEDKFSII
Q99RS1              KIEIQNALVDLMA-EYPFQEISTKMICAYCNINRSTFYDYYKDKFDLL

                        ————— α₁ —————           — α₂ —    — α₃ —  — α₄ →
QacR                KDKILGVAKELFI-KNGYNATTTGEIVKLSESSKGNLYYHFKTKENLF
                    |----+----+- ---+----+----+----+----+----+----|
                    4          10          20          30          40    50
```

FIG. 1. Alignment of 42 members of the TetR family that exemplify the TetR family profile. The blue column indicates α-helix residues involved in DNA contacts in the crystal structure of TetR and QacR. The yellow column indicates turns. The most conserved residues are shaded. Abbreviations are as follows: BACME, *Bacillus megaterium*; BACSU, *Bacillus subtilis*; ECOLI, *Escherichia coli*; HAEIN, *Haemophilus influenzae*; LACLA, *Lactobacillus lactis*; MYCTU, *Mycobacterium tuberculosis*; RHIME, *Rhizobium meliloti*; STRGA, *Streptomyces* sp.; VIBHA, *Vibrio haemophilus*; XANAU, *Xanthomonas* sp.
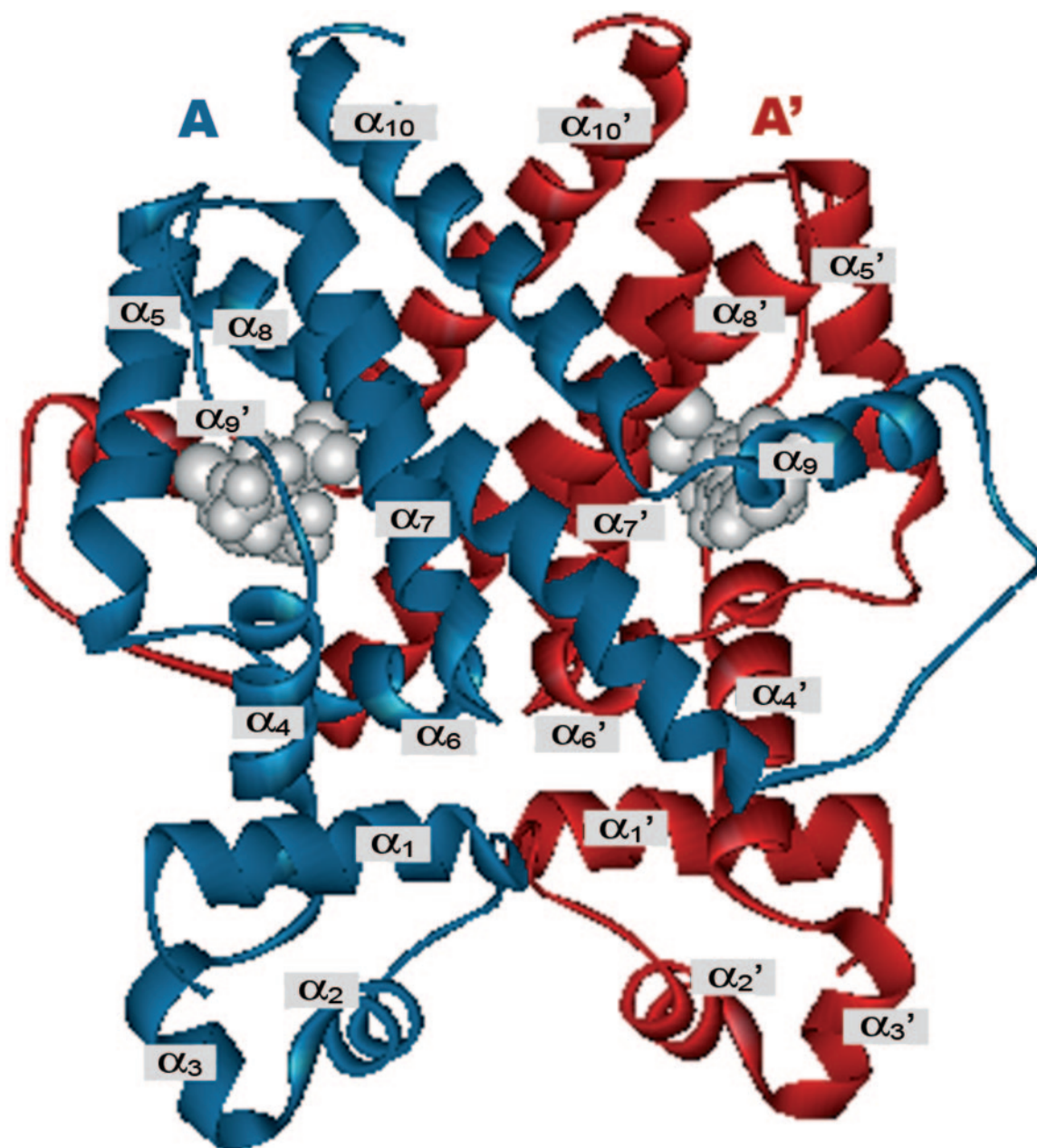
FIG. 2. Ribbon diagram of a TetR homodimer. Monomers are shown in blue or red. Two tetracycline molecules, each bound to a monomer, are shown in grey. α-Helices 2 and 3 in the blue monomer and α2′ and α3′ in the red monomer constitute the shared HTH DNA binding domain. α-Helix 1 and part of helix α-4, together with α-helices 2 and 3, comprise the sequence that best defines the TetR family profile. (Adapted from Hinrichs et al. [150] with permission of the publisher.)

2004) using the pfsearch program available at http://bioweb .pasteur.fr/seqanal/interfaces/pftools.html#pfsearch (46). The program, which proposes a tentative threshold Z-score of 8.5 to consider a protein a member of the TetR family, selected 2,357 proteins as putative members of the TetR family.

To verify the quality of this TetR profile for specificity (false positives) and sensitivity (false negatives), we implemented a new tool called Provalidator which uses Interpro, Swiss-Prot, Prodom, TIGRfam, CoGnitor, NCBI-RPS-BLAST, and PSI-BLAST resources (68, 128, 154, 323, 348, 387, 449). In the first step, we searched for false positives among the 2,353 proteins

we assigned to the TetR family. Interpro assigned 2,315 proteins to the TetR family, and these 2,315 were considered true positives. The remaining 38 proteins were analyzed with other resources such as TIGRfam, Prodom, NCBI-RPS-BLAST and PSI-BLAST (128, 449). This allowed us to assign 34 proteins to the TetR family. Three of the false positives (Q89RN6, Q988I6, and Q6N8G8) that we found were protein members of the AraC/XylS family of transcription activators (109, 394). These proteins have two HTH motifs at the C-terminal end, typical of AraC/XylS family members (109, 229). These three proteins were identified as potential TetR members because

one of its HTH is highly similar to the DNA-binding domain in TetR. The fourth false positive is a transposase (Q981E7).

Provalidator detected 15 false negatives (Q742Y2, Q8CJK3, Q73ZY1, Q6D1J7, Q8KU64, Q9A917, Q880T2, Q6D2Z4, Q885G7, Q8PC90, Q9A466, Q9S6C0, Q9ZH26, Q6A626, and Q8G822), which are proteins assigned to the TetR family by INTERPRO but whose Z-score was between 6.407 and 8.487. In summary, the TetR profile with a Z-score threshold of 8.5 identified proteins that were not detected by INTERPRO, and among the 660,992 proteins analyzed, only four false positives were found. These results indicate that the new algorithm is highly effective for the detection of members of the TetR family.

Identification of TetR Family Members in DNA and Protein Databases

Using the profile defined above for the TetR family, we searched for members of this family in the Swiss-Prot and TrEMBL databases and also searched the 196 complete and incomplete microbial genomes available in NCBI (Release December 2004). We detected 73 TetR proteins in Swiss-Prot, 2,277 in TrEMBL, and 2,410 in the translated open reading frame corresponding to 196 microbial genomes. To select non-redundant sequences the set of 4,758 TetR proteins was analyzed using the SEQUNIQ program developed in our laboratory (Molina-Henares et al., unpublished results). This program integrates the set of sequences available in nucleic acid and protein databases. We found 2,353 sequences in the TetR family that surpassed the threshold Z-score of 8.5. The HTH in 2,348 members of the family was located at the N-terminal end of the proteins.

Table 3 shows that members of the TetR family were detected in 144 microbial genomes belonging to 80 genera and 113 species of gram-positive and α-, β-, and γ-proteobacteria, cyanobacteria, and archaea, indicating wide taxonomic distribution. We have found that proteins of the TetR family are encoded both in chromosomes and in plasmids, and the mobility of the latter elements could be a source of the spread of genes in this family via horizontal transfer (147, 383), as is also the case with catabolic genes (77, 160, 236, 410, 426), antimicrobial resistance determinants (20, 100, 124), and 16S rRNA genes (347).

We found that TetR family members are particularly abundant in microbes exposed to environmental changes, such as soil microorganisms (i.e., *Nocardia*, *Streptomyces*, *Bradyrhizobium*, *Mesorhizobium*, *Pseudomonas*, *Bacillus*, and *Ralstonia* spp.); plant and animal pathogens (i.e., *Agrobacterium*, *Brucella*, *Escherichia coli*, *Bordetella*, *Mycobacterium*, and *Salmonella* spp.), extremophiles (i.e., *Deinococcus*), and methanogenic bacteria such as *Methanosarcina acetivorans*. In contrast, TetR family members do not appear in intracellular pathogens such as chlamydias, mycoplasmas, and endosymbionts such as *Buchera*, in agreement with their life style in nonchanging environments (52). However, it should be noted that Dugan et al. (80) recently found that *Chlamydia suis* can acquire tetracycline resistance via horizontal gene transfer of genomic islands bearing the *tet* genes.

As a general collorarium, we can say that it seems that proteins of the TetR family are involved in the adaptation to complex and changing environments. This in turn correlates with the fact that many members of the TetR family are found among microbes with abundant extracytoplasmic function sigma factors (52, 227, 236, 277, 444).

PROTEINS WITH KNOWN THREE-DIMENSIONAL STRUCTURES

The high degree of primary sequence identity in the stretch that defines the HTH region of the TetR profile probably reflects a common three-dimensional structure in this domain in members of the family. This is supported by the almost identical three-dimensional structure of the HTH of TetR, QacR, CprB, and EthR, as deduced from the superimposition of these regions, and the high degree of sequence conservation in the alignment (79, 264, 349). As in other families of transcriptional regulators, no sequence conservation was found outside the HTH domain, which probably reflects differences in the kind of signal sensed by different regulators of the family, i.e., antibiotics with dissimilar structures, barbiturates, homoserine lactones, organic solvents, and choline (see Table 2). Nonetheless, some striking global structural conservation in the three-dimensional structure was found.

In addition, given that all members of the family whose function is known are repressors, they probably function in a similar way. Binding of an inducer molecule to the nonconserved domain of a TetR family member probably causes conformational changes in the conserved DNA-binding region that result in release of the repressor from the operator and thus allow transcription from the cognate promoter. To gain insights into the mechanisms of action of the TetR family members, we analyzed in detail the three-dimensional structure of the four members of the family, TetR, QacR, CprB, and EthR, whose crystal structures have been obtained (150, 264, 286–289, 349–351), in order to identify common and differential features of the TetR family members.

TetR Regulator

Tetracycline resistance and the role of the transcriptional regulator TetR. Tetracyclines are among the most commonly used broad-spectrum antibiotics (209, 210). They act by binding to the small ribosomal subunit, thereby interrupting polypeptide chain elongation by an unknown mechanism. Many gram-negative bacteria have developed mechanisms of resistance against this antibiotic. The most frequent mechanism involves a membrane-associated protein (TetA) that exports the antibiotic out of the bacterial cell before it inhibits polypeptide elongation (169, 211, 389, 434, 435, 453).

Adjacent to *tetA* and divergently oriented is *tetR* (112), whose gene product tightly controls expression of both *tetA* and *tetR* (148, 150). The intergenic region between the *tetR* and *tetA* genes contains two identical operators separated by 11 bp. TetR binds to these operators and thus prevents transcription from both promoters (Fig. 3) and (288). In all TetR crystal structures elucidated to date (PDB identifiers: 2TCT; 2TRT; 1A6I; 1BJO; 1BJY; 1BJZ; 1ORK; and 1RP1), this repressor appears as a homodimer (29, 30, 159, 183, 287–289, 366). The TetR homodimer binds to the operator (Fig. 3). Each 15-bp operator shows an internal palindromic symmetry with an extra

TABLE 3. Distribution of TetR proteins in microbes

| Microorganism	Genome size (Mbp)	No. of members	Microorganism	Genome size (Mbp)	No. of members
Nocardia farcinica IFM 10152	6.21	151	*Shigella flexneri* 2a 301	4.61	12
Streptomyces coelicolor A3(2)	9.05	150	*Vibrio cholerae* O1 biovar eltor N16961	4.03	12
Streptomyces avermitilis MA-4680	9.12	116	*Nostoc* sp. strain PCC 7120	7.21	11
Mycobacterium avium subsp. *paratuberculosis* k10	4.83	108	*Enterococcus faecalis* V583	3.22	10
Agrobacterium tumefaciens C58	11.35	61	*Mycobacterium leprae* TN	3.27	10
Bradyrhizobium japonicum USDA 110	9.11	59	*Shigella flexneri* 2a 2457T	4.60	10
Mycobacterium bovis AF2122/97	4.35	51	*Geobacter sulfurreducens* PCA	3.81	9
Mycobacterium tuberculosis CDC1551	4.40	51	*Leptospira interrogans* serovar Copenhageni Fiocruz L1-130	4.63	9
Mycobacterium tuberculosis H37Rv	4.41	51	*Leptospira interrogans* serovar Lai 56601	4.69	9
Bacillus licheniformis ATCC 14580	8.44	48	*Propionibacterium acnes* KPA171202	2.56	9
Mesorhizobium loti MAFF303099	7.60	47	*Staphylococcus aureus* subsp. *aureus* MRSA252	2.90	9
Rhodopseudomonas palustris CGA009	5.46	40	*Bifidobacterium longum* NCC2705	2.26	8
Pseudomonas aeruginosa PAO1	6.26	38	*Brucella melitensis* 16M	3.29	8
Bacillus cereus ATCC 10987	5.22	36	*Brucella suis* 1330	3.32	8
Bacillus anthracis 'Ames Ancestor'	5.50	32	*Photorhabdus luminescens* subsp. *laumondii* TTO1	5.69	8
Bacillus anthracis A2012	5.37	32	*Staphylococcus aureus* subsp. *aureus* Mu50	2.90	8
Bacillus anthracis Sterne	5.23	31	*Symbiobacterium thermophilum* IAM 14863	3.57	8
Bacillus anthracis Ames	5.23	30	*Yersinia pestis* biovar Medievalis 91001	4.80	8
Bacillus cereus ZK	5.30	30	*Yersinia pseudotuberculosis* IP 32953	4.84	8
Bacillus cereus ATCC 14579	5.43	29	*Bacteroides fragilis* YCH46	5.31	7
Bordetella bronchiseptica RB50	5.34	28	*Bacteroides thetaiotaomicron* VPI-5482	6.26	7
Pseudomonas syringae pv. tomato DC3000	6.40	28	*Bdellovibrio bacteriovorus* HD100	3.78	7
Sinorhizobium meliloti 1021	6.69	28	*Desulfovibrio vulgaris* subsp. *vulgaris* Hildenborough	3.77	7
Bacillus thuringiensis serovar konkukian 97-27	5.24	27	*Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586	2.17	7
Clostridium acetobutylicum ATCC 824	4.13	27	*Staphylococcus aureus* subsp. *aureus* MSSA476	2.80	7
Caulobacter crescentus CB15	4.02	26	*Staphylococcus aureus* subsp. *aureus* MW2	2.82	7
Lactobacillus plantarum WCFS1	3.31	26	*Staphylococcus aureus* subsp. *aureus* N315	2.84	7
Pseudomonas putida KT2440	6.18	25	*Yersinia pestis* CO92	4.83	7
Burkholderia pseudomallei K96243	7.25	24	*Desulfotalea psychrophila* LSv54	3.66	6
Ralstonia solanacearum GMI1000	5.81	24	*Lactobacillus johnsonii* NCC 533	1.99	6
Photobacterium profundum SS9	6.40	23	*Mannheimia succiniciproducens* MBEL55E	2.31	6
Oceanobacillus iheyensis HTE831	3.63	22	*Rhodopirellula baltica* SH 1	7.15	6
Bordetella parapertussis 12822	4.77	21	*Streptococcus agalactiae* NEM316	2.21	6
Burkholderia mallei ATCC 23344	5.84	21	*Yersinia pestis* KIM	4.60	6
Bacillus halodurans C-125	4.20	20	*Aquifex aeolicus* VF5	1.59	5
Bacillus subtilis subsp. *subtilis* 168	4.21	20	*Methylococcus capsulatus* Bath	3.30	5
Acinetobacter sp. strain ADP1	3.60	19	*Streptococcus agalactiae* 2603V/R	2.16	5
Bordetella pertussis Tohama I	4.09	17	*Streptococcus pyogenes* MGAS10394	1.90	5
Chromobacterium violaceum ATCC 12472	4.75	17	*Streptococcus pyogenes* MGAS8232	1.90	5
Shewanella oneidensis MR-1	5.13	17	*Clostridium perfringens* 13	3.09	4
Vibrio vulnificus CMCP6	5.13	17	*Methanococcus maripaludis* S2	1.66	4
Escherichia coli CFT073	5.23	16	*Staphylococcus epidermidis* ATCC 12228	2.50	4
Gloeobacter violaceus PCC 7421	4.66	16	*Streptococcus pyogenes* M1 GAS	1.85	4
Methanosarcina acetivorans C2A	5.75	16	*Streptococcus pyogenes* MGAS315	1.90	4
Streptococcus mutans UA159	2.03	16	*Streptococcus pyogenes* SSI-1	1.89	4
Vibrio parahaemolyticus RIMD 2210633	5.17	16	*Thermus thermophilus* HB27	2.13	4
Vibrio vulnificus YJ016	5.26	16	*Clostridium tetani* E88	2.80	3
Xanthomonas axonopodis pv. citri 306	5.18	16	*Haemophilus influenzae* Rd KW20	1.83	3
Corynebacterium glutamicum ATCC 13032	3.31	15	*Methanosarcina mazei* Go1	4.10	3
Deinococcus radiodurans R1	3.28	15	*Nitrosomonas europaea* ATCC 19718	2.81	3
Erwinia carotovora subsp. *atroseptica* SCRI1043	5.06	15	*Pasteurella multocida* subsp. *multocida* Pm70	2.26	3
Xanthomonas campestris pv. campestris ATCC 33913	5.08	15	*Porphyromonas gingivalis* W83	2.34	3
Escherichia coli O157:H7	5.59	14	*Streptococcus pneumoniae* R6	2.04	3
Corynebacterium efficiens YS-314	3.15	13	*Streptococcus pneumoniae* TIGR4	2.16	3
Escherichia coli O157:H7 EDL933	5.53	13	*Synechocystis* sp. strain PCC 6803	3.57	3
Lactococcus lactis subsp. *lactis* Il1403	2.37	13	*Thermoanaerobacter tengcongensis*	2.69	3
Leifsonia xyli subsp. *xyli* CTCB07	2.58	13	*Wolinella succinogenes* DSM 1740	2.11	3
Listeria monocytogenes 4b F2365	2.91	13	*Haemophilus ducreyi* 35000HP	1.70	2
Salmonella enterica subsp. *enterica* serovar Typhi CT18	5.13	13	*Halobacterium salinarum* NRC-1	2.57	2
Salmonella typhimurium LT2	4.95	13	*Legionella pneumophila* str. Lens	3.41	2
Treponema denticola ATCC 35405	2.84	13	*Legionella pneumophila* str. Paris	3.64	2
Corynebacterium diphtheriae NCTC 13129	2.49	12	*Legionella pneumophila* subsp. *pneumophila* Philadelphia 1	3.40	2
Escherichia coli K12	4.64	12	*Methanothermobacter thermautotrophicus* Delta H	1.75	2
Listeria innocua Clip11262	3.01	12	*Neisseria meningitidis* MC58	2.27	2
Listeria monocytogenes EGD-e	2.94	12	*Neisseria meningitidis* Z2491	2.18	2
Salmonella enterica subsp. *enterica* serovar Typhi Ty2	4.79	12	*Thermotoga maritima* MSB8	1.86	2
Shigella flexneri 2a 301	4.61	12	*Xylella fastidiosa* 9a5c	2.73	2
Vibrio cholerae O1 biovar eltor N16961	4.03	12	*Archaeoglobus fulgidus* DSM 4304	2.18	1
Nostoc sp. strain PCC 7120	7.21	11	*Campylobacter jejuni* subsp. *jejuni* NCTC 11168	1.64	1
Enterococcus faecalis V583	3.22	10	*Coxiella burnetii* RSA 493	2.00	1
Mycobacterium leprae TN	3.27	10	*Helicobacter hepaticus* ATCC 51449	1.80	1
Shigella flexneri 2a 2457T	4.60	10	*Mycoplasma penetrans* HF-2	1.36	1
Geobacter sulfurreducens PCA	3.81	9	*Picrophilus torridus* DSM 9790	1.55	1
Leptospira interrogans serovar Copenhageni Fiocruz L1-130	4.63	9	*Pyrococcus abyssi* GE5	1.77	1
Leptospira interrogans serovar Lai 56601	4.69	9	*Sulfolobus solfataricus* P2	2.99	1
Propionibacterium acnes KPA171202	2.56	9	*Sulfolobus tokodaii* 7	2.69	1
Staphylococcus aureus subsp. *aureus* MRSA252	2.90	9	*Ureaplasma parvum* serovar 3 ATCC 700970	0.75	1
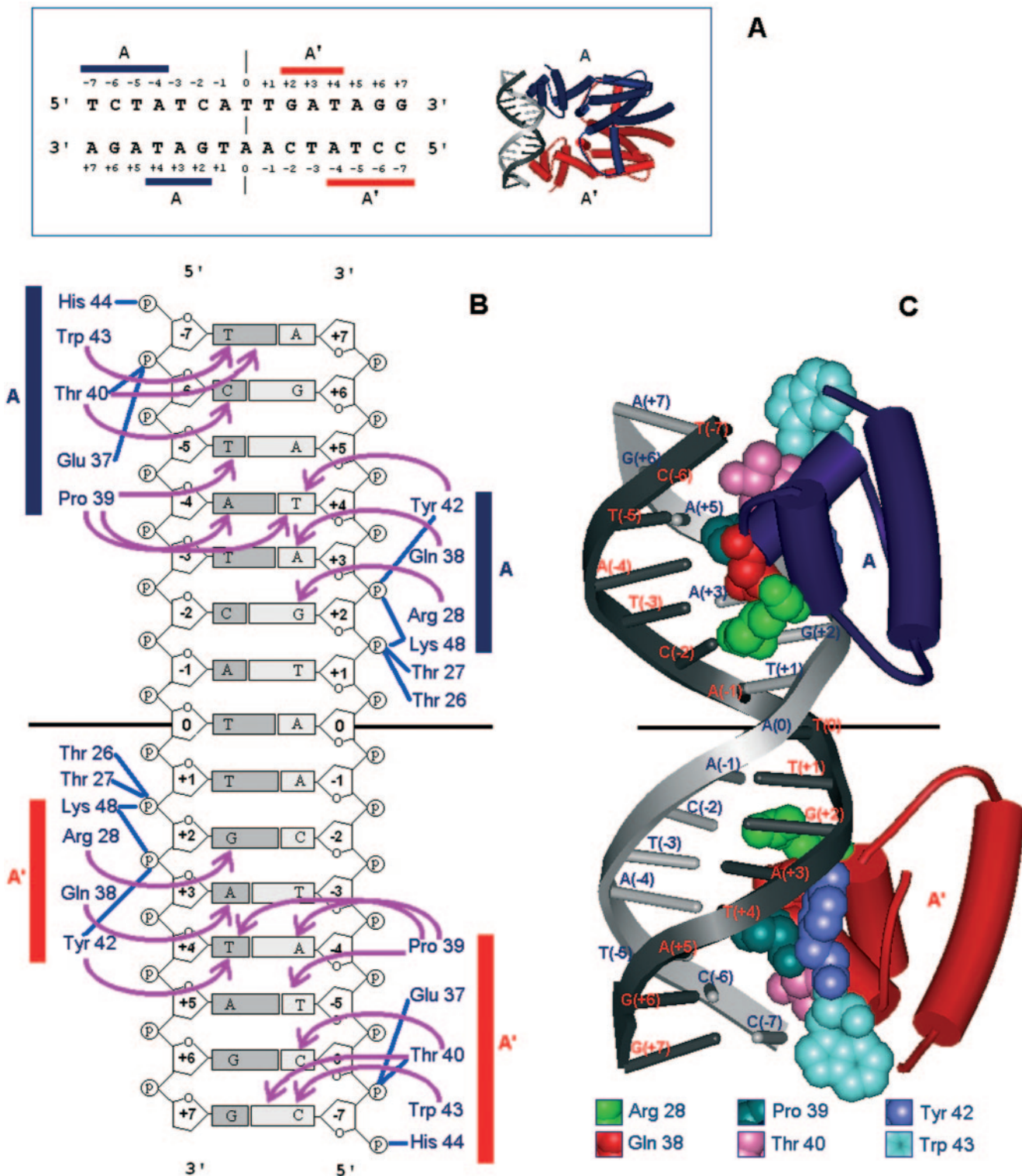
FIG. 3. Binding of TetR to its operator site. A) *tetR* operator and contact regions. The *tetR* operator is a palindromic sequence. Horizontal bars show nucleotides contacted by each monomer of the TetR dimer. B) Interaction of TetR residues with specific nucleotides (arrows) and phosphate backbone (blue lines) in the operator region. The amino acids involved in DNA binding extend from residues 27 to 48. Contacts established with the operator were confirmed by footprint assays, by analysis of TetR mutants, and by crystallographic studies (29, 30, 159, 266, 366). C) Representation of each homodimer bound to the *tet* operator in a double-helix representation. (Adapted from Orth et al. [288] with permission of the publisher.)
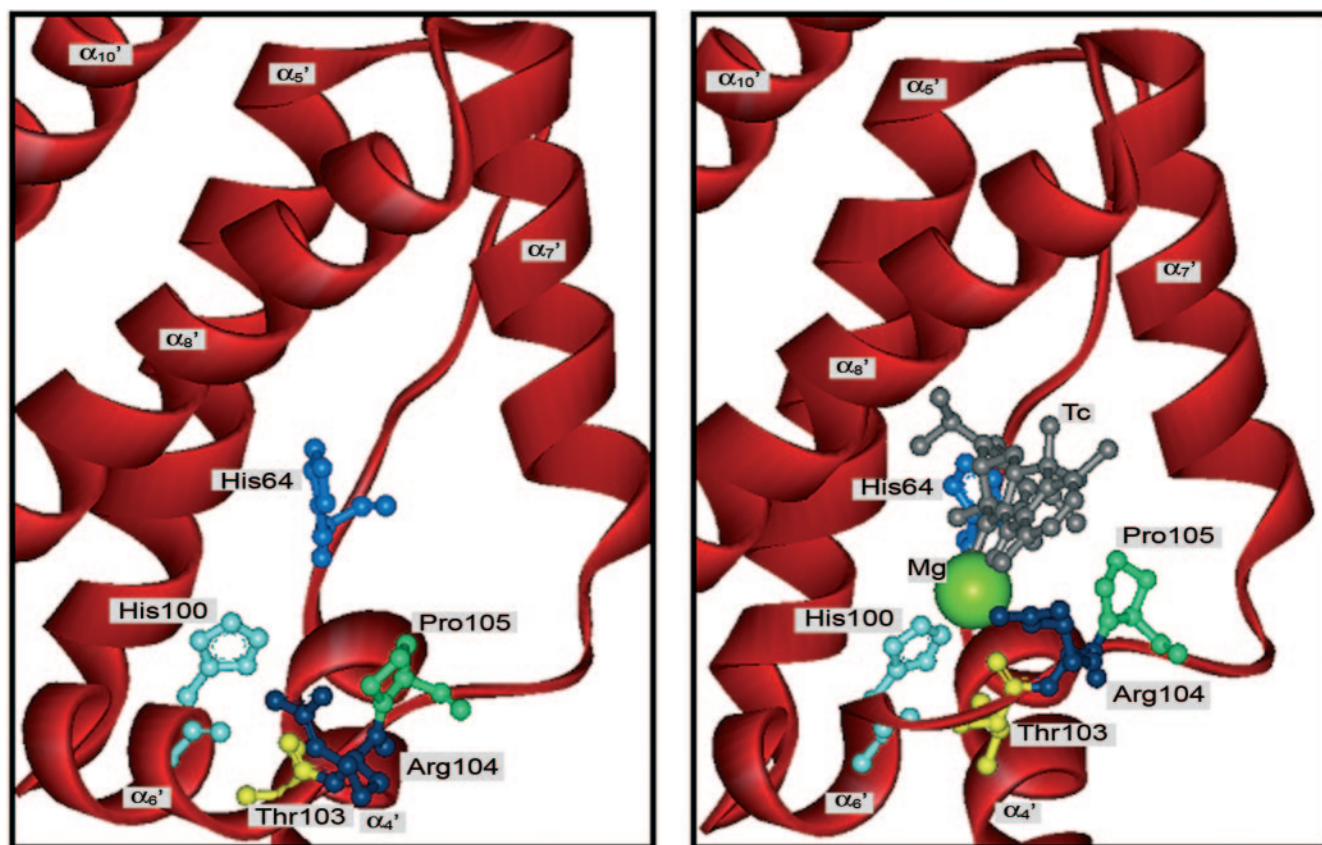
FIG. 4. Representation of the TetR cavity involved in the binding of tetracycline. Left) In the absence of tetracycline. Right) In the presence of tetracycline. The green ball represents the Mg^{2+} ion. Specific interactions are not drawn for the sake of clarity but are described in the text. (Adapted from Orth et al. [287, 289] and Kisker et al. [183] with permission of the publishers.)

central base pair (Fig. 3A). The operator sequences overlap with promoters for *tetA* and *tetR*, thereby blocking the expression of both genes. When tetracycline complexed with Mg^{2+} binds to TetR (166, 384), a conformational change takes place that renders the TetR protein unable to bind DNA. As a consequence, TetR and TetA are expressed (286).

The TetR homodimer is constituted by two identical monomers that fold into 10 α-helices with connecting turns and loops (Fig. 2). The three-dimensional structure of the TetR monomer is stabilized mainly by hydrophobic helix-to-helix contacts. The global structure of the TetR homodimer can be divided into two DNA-binding domains at the N-terminal end of each monomer, and a regulatory core domain involved in dimerization and ligand binding (150, 286–289). The DNA-binding domains are constituted by helices α1, α2, and α3 and their symmetric helices α1′, α2′, and α3′ (a prime denotes the second monomer). Helices α4 and α4′ connect these domains with the regulatory core domain composed of helices α5 to α10 and their symmetric counterparts α5′ and α10′ (150, 287, 289). The regulatory domain is responsible for dimerization and contains, for each monomer, a binding pocket that accommodates tetracycline in the presence of a divalent cation. Helices α5, α8, and α10 and their counterparts α5′, α8′, and α10′ constitute the scaffold of the core domain, and their structure is the most conserved in both TetR conformations (150, 287–289).

The tetracycline-binding pocket is identical in both mono-

mers. The cavity to which the $[TcMg]^+$ complex binds is depicted in Fig. 4 (286, 287, 289). The entrance of this cavity is controlled by α9′ and the C-terminal end of α8′ and the loop that connects both, while the exit is closed by loop 4-5 (287–289). When $[TcMg]^+$ enters the tunnel, its A ring makes contacts with loop 4-5, and the interaction with the effector triggers a cascade of conformational changes. The contacts that His100 and Thr103, both in α6, establish with the magnesium ion of the complex displace α6, which undergoes a conformational change in its C terminus to form a β-turn (Fig. 4). The 6-7 loop is also pushed near the inducer, so that Arg104 and Pro105 interact with tetracycline. Translation of α6 forces α4 to move in the same direction due to van der Waals contacts. His64 of α4, anchored to α5 and to tetracycline, acts as a pivot point, and α4 moves like a pendulum. As a consequence of the rotation of α4 and α4′, recognition helices α3 and α3′ move further apart, and the DNA contacts are disrupted (Fig. 5) (286, 287, 289). Tetracycline is impeded from freeing the binding cavity, and TetR cannot bind its target DNA again. It should be noted that residues outside the binding cavity can influence affinity for tetracycline, as revealed by Kamionka et al. (168), who isolated a double mutant (G96E, L205S) with reduced affinity for the antibiotic.

The on/off switch mechanism used by TetR to respond to specific signals may be used similarly in other TetR family members.

FIG. 5. Docking of a TetR monomer without (grey) and with (red) tetracycline. Note the alterations induced in the α_1-α_3 region involved in binding to the target operators. The increase in distance between $\alpha3$ and $\alpha3'$ with tetracycline results in the inability of TetR to maintain the specific interactions shown in Fig. 3, and therefore the repressor is released. (Adapted from Orth et al. [288] with permission of the publisher.)

TetR DNA-binding domain: a symmetric TetR dimer binds a palindromic operator. Cocrystallization of TetR with its operator DNA established that the TetR homodimer binds perpendicularly to the longitudinal DNA axis (Fig. 3A). Two adjacent DNA major groove regions covering a 6-base-pair area on both strands are involved in the almost perfect docking with the two TetR-interacting domains (Fig. 3A and 3B) (288). No water molecules were found at the TetR-DNA interface, where the crucial interactions are hydrophobic (288).

The interactions of each HTH domain with the operator DNA are summarized in Fig. 3A and 3B. The TetR monomer A binds the main strand from positions −4 to −7 while contacting the complementary strand from operator positions +4 to +2, and the symmetric monomer A' binds the main strand from positions +2 to +4 and the complementary strand from positions −4 to −7 (Fig. 3A and 3B).

Crystallographic analysis revealed that helix $\alpha3$ (from Gln38 to His44) is the main element responsible for sequence-specific recognition, since all residues in this helix contribute to it, except for Leu41, which is part of the hydrophobic core stabilizing the $\alpha1$, $\alpha2$, and $\alpha3$ helix bundle. Thr40 residue in monomer A establishes direct contacts with operator base pairs

T(−7) and C(−6) in the main DNA strand (Fig. 3A and 3B). Trp43 interacts with T(−7) as well. Pro39 interacts with both strands at bases T(−5) and A(−4) of the main strand and T(+4) of the complementary strand. In the rest of the operator half site, the $\alpha3$ helix of monomer A interacts with the complementary strand, Tyr42 contacting with T(+4) and Gln38 with A(+3). Helix $\alpha2$ supplies an additional specific contact with the complementary strand, namely, Arg28 contacts G(+2).

Although the TetR DNA binding domain maintains its structure thanks to a hydrophobic core formed by residues from the $\alpha1$, $\alpha2$, and $\alpha3$ bundle (288), interactions with DNA lead to changes in the TetR DNA binding domain. One such change is that $\alpha3$ forms a 3_{10}–helical turn at the N-terminal end as a result of complex DNA contacts. The H-bonds between Arg28-G(+2), and Gln38-A(+3) increase the separation between base pairs 1 and 2 from 3.4 Å to 3.9 Å (288). The two phosphate groups accompanying the G at position +2 establish H-bonds with side chains of Thr26, Thr27, Tyr42, and Lys48, and with the amino groups of the main chain of Thr27 and Lys48 (Fig. 3B). These contacts draw DNA closer to TetR near G(+2). Although the DNA is kinked away from TetR at position +2 in both operator strands, bending toward TetR in
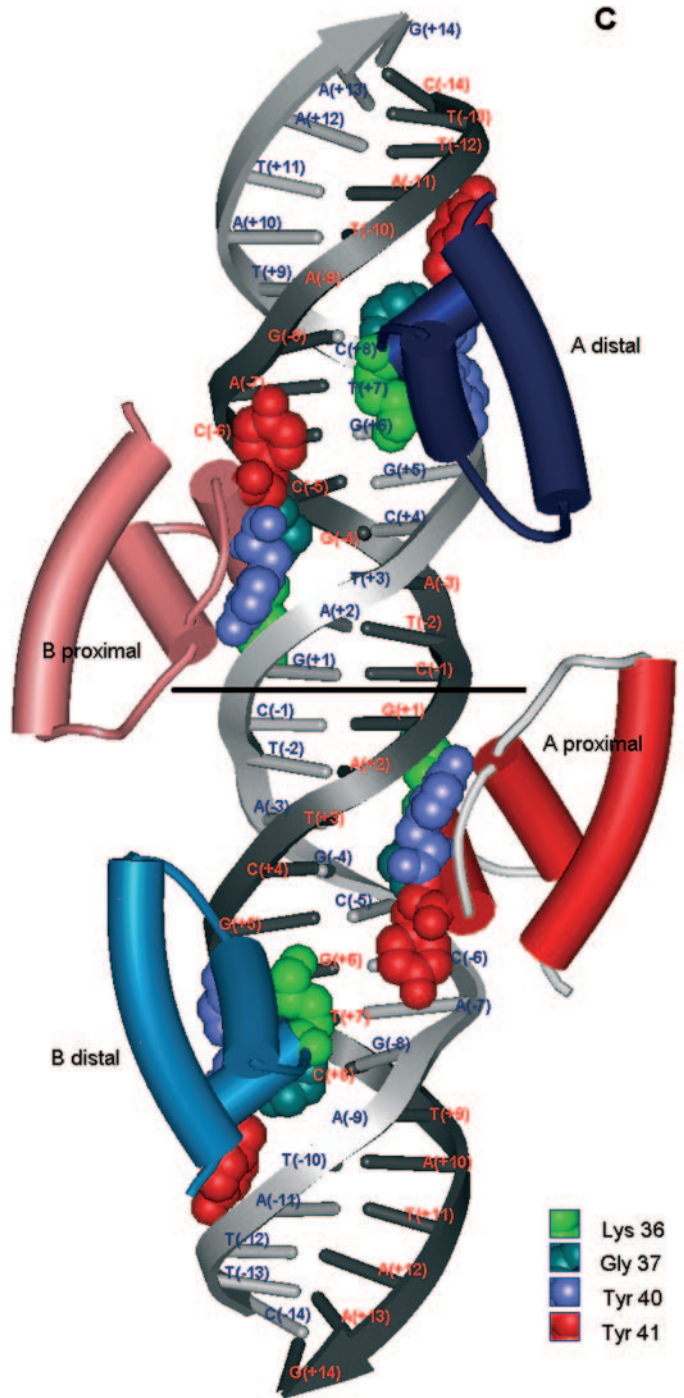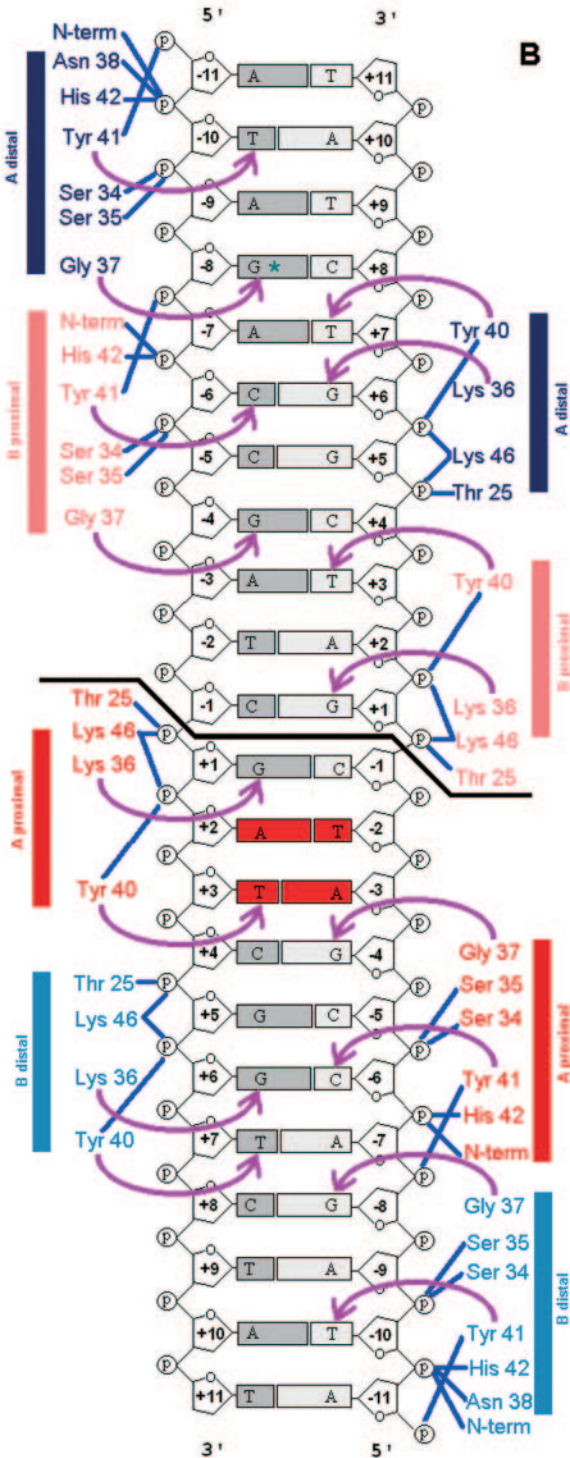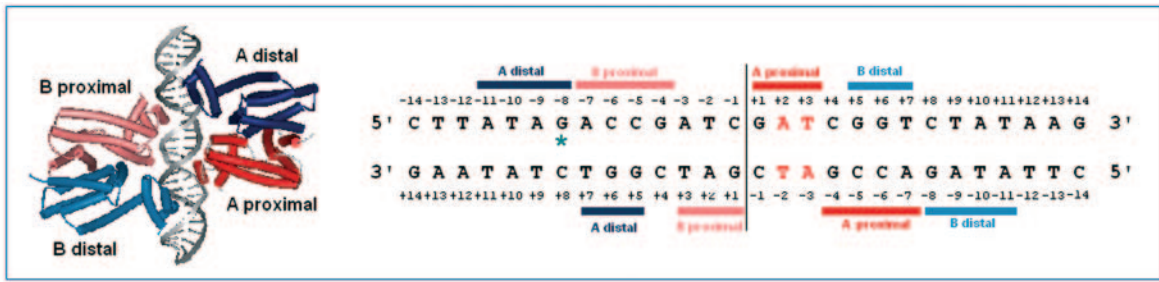
the area corresponding to positions +3 to +6 compensates for the DNA deviation. Crystallographic studies revealed that Lys48 located in α4, outside the HTH motif, also established contacts with the target DNA region (Fig. 3B). This lysine is relatively well conserved among TetR family members, and we are tempted to suggest that this residue plays an equivalent role in other proteins of the TetR family.

QacR Regulator

Two QacR dimers bind the operator to repress the *qacA* multidrug transporter gene. QacA confers resistance to monovalent and bivalent cationic lipophilic antiseptics and disinfectants such as *q*uaternary *a*mmonium *c*ompounds (hence the name Qac) (10, 11, 44, 239). The *qac* locus consists of the *qacA* gene and the divergently transcribed *qacR* gene, which are borne on a plasmid (119). In the absence of drug, the 188-residue QacR protein represses transcription of the *qacA* multidrug transporter gene by binding two nested palindromes located downstream from the *qacA* promoter and overlapping its transcription start site (119, 300). Therefore, QacR seems to repress transcription by hindering the transition of the RNA polymerase-promoter complex into a productively transcribing state rather than by blocking RNA polymerase binding.

The three-dimensional structure of QacR (PDB identifiers 1JTX, 1JTG, 1JTY, 1JUM, 1JUP, 1JUS, and 1JTO) revealed that it is an all-helical protein which contains a DNA-binding HTH motif embedded within an N-terminal three-helix bundle and a second domain involved in drug binding and dimerization (350, 351). It should be noted that unlike TetR, two QacR dimers, rather than one, bind the operator site (339, 340) (Fig. 6).

The monomers of each dimer have been called proximal and distal to refer to their positions with respect to the center of symmetry of the palindromic operator (Fig. 6A and 6B). It was shown that the operator to which one dimer is bound is symmetric and partially overlaps that bound by the other dimer (351) (Fig. 6 and 7). The existence within the same fragment of DNA sequence of two overlapping partial palindromes with identical symmetric bases is therefore surprising (Fig. 6). In this sense the palindromic sequences recognized by QacR are equivalent to those described for the TetR interface except for the spacer sequence length, 3 bp for TetR versus 4 bp for QacR, supporting the hypothesis that interactions of other members of the family with their target sequences may be similar, independent of the number of dimers involved.

The α3 helix of QacR A distal and B distal monomers establish the most extensive specific interactions with the operator (351). The Tyr41 residue of the A distal monomer (Fig. 6B) establishes hydrophobic contacts with base T(−10) of the DNA main strand as well as with the phosphate at position −11 in the main strand, while Tyr40 contacts T(+7) (Fig. 6B). In addition, tight docking with DNA is facilitated by specific hydrogen bonds between Lys36 and base G(+6) in the com-

plementary strand, and between Gly37 and base G(−8) in the main strand. Gly37 is important in repression because nucleotide G(−8) is the transcription start site for the *qacA* gene. Monomers A and B proximal also establish a series of critical interactions. For instance, Tyr41 of B proximal contacts the C(−6) base in the main strand, whereas Tyr40 contacts base T(+3) and phosphate (+2) in the complementary strand (351). Gly37 in the A proximal monomer contacts G(−4) in the complementary strand, whereas Lys36 contacts G(+1) in the main strand. A number of residues in α2, loop α2-α3, α3 and the positive dipole of the α1 (N terminus) also interact with the phosphate backbone of both DNA strands (351).

Figure 6C shows how each dimer engages the DNA major groove in a face almost opposite to the other dimer, forming an angle between the two dimer axes of less than 180° (Fig. 7). Studies of QacR binding to DNA have indicated that the two dimers bind DNA cooperatively (120, 121, 351). Analysis of the three-dimensional structure suggested that such cooperativity does not arise from protein-protein interactions, as the closest approach of the dimers is 5.0 Å. Rather, binding cooperativity appears to be mediated through conversion of the DNA structure from a B-DNA conformation to the high-affinity undertwisted configuration observed in the crystal structure. Conversion of the DNA conformation is necessary because the optimal distance between each of the HTH motifs of the QacR dimer is 37 Å. This requires expansion of the 34-Å distance between successive major groove regions on one edge of the canonical B-DNA. It has been suggested that binding of the first QacR dimer forces this energetically unfavorable conformational change, which in turn produces an optimal DNA conformation for the easy binding of the second dimer (351). Experimental data reported by Grkovic et al. (121, 122) suggested that the two dimers must bind simultaneously and cooperatively to the operator in order to maintain the DNA deformation detected in the crystal.

Schumacher and Brennan (349) noticed that TetR and QacR achieve the same degree of specificity in DNA binding through different mechanisms. They noted that TetR, recruits Arg28, located outside its recognition helix, to make a base pair-specific contact (288), whereas QacR does not employ residues outside α3 to ensure DNA binding specificity. They also noted that TetR kinks its binding site and induces a 17° bend towards the protein to optimize the position of its HTH motifs for specific base interactions within each DNA half site; whereas QacR widens the major groove of the entire IR1 binding site smoothly and bends its DNA site by only 3°. These distinctions are reflected in the different HTH center-to-center distances observed in QacR (37 Å). Thus, an important lesson derived from comparisons of the QacR-DNA and TetR-DNA structures is that even structurally homologous proteins of the same family that share a similar function, i.e., repression, can utilize slightly different mechanisms of action.

FIG. 6. Binding of QacR to its operator site. A) Interaction of QacR with the *qac* operator. B) Contacts established by residues at α-helix 3 of QacR homodimers A and B with specific nucleotides (arrows) and phosphate backbone (blue lines) in the synthetic operator used for QacR-DNA cocrystal (349, 350). C) Representation of the two QacR homodimers bound to the *qac* operator in a double-helix representation. (Adapted from Schumacher et al. [351] with permission of the publisher.)
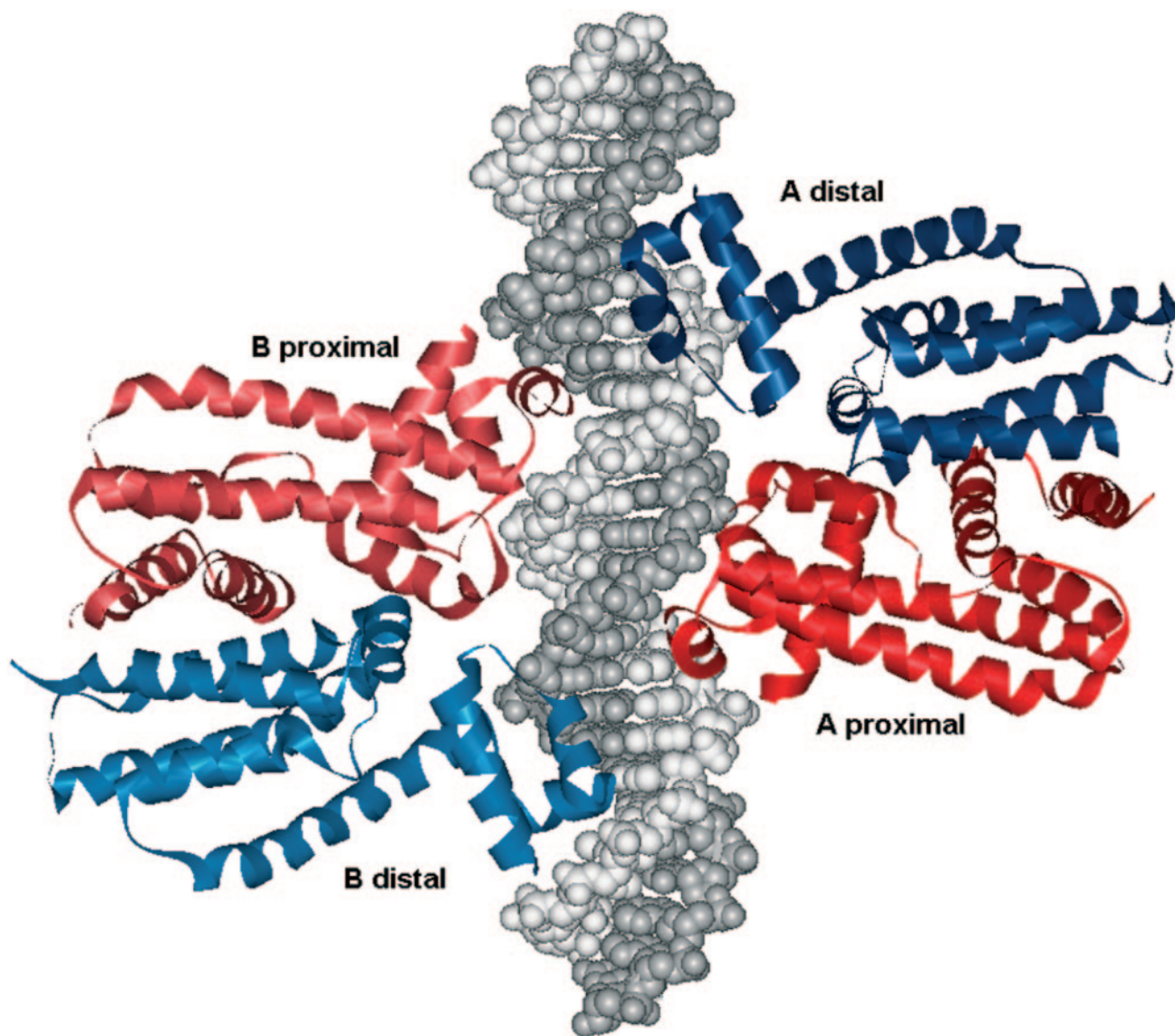
FIG. 7. Ribbon representation of the two QacR homodimers bound to target DNA in a double-helix representation (A) and details of the contacts established by α-helix 3 of monomers of different homodimers when recognizing overlapping sites (B). (Adapted from Schumacher et al. [351] with permission of the publisher.)

QacR as a model for multidrug recognition. QacR is released from the *qacA* operator by its interaction with a number of cationic lipophilic drugs such as rhodamine 6G, crystal violet, and ethidium (119). More recently, Grkovic et al. (122) showed that effector recognition of QacR can be extended to several bivalent cationic dyes and plant alkaloids. In spite of the existence of two binding pockets, only one drug molecule is bound by each homodimer, as determined by equilibrium dialysis studies and isothermal titration calorimetry for the QacR-R6G complex (350). The QacR crystal bound to different drugs revealed another remarkable finding: the presence of an expansive and multifaceted drug-binding pocket with a volume of 1,100 Å^3, so that different drugs partially overlap different subpockets (349, 351). A similar cavity able to bind multiple drugs was reported by Yu et al. (445, 446) for the AcrB multidrug transporter.

Crystallographic studies by Schumacher et al. (350) and

Murray et al. (261) have demonstrated that multidrug recognition mediated by the QacR dimer is a rather simple process that, contrary to expectations, does not require sophisticated molecular mechanisms. Indeed, the drug binding domain of QacR consists of six α-helices (PDB identifiers: 1JTX, 1JT6, 1JTY, 1JUP, 1JUS, 1JTO, 1RKW, and 1RPW). Entry to the mostly buried drug-binding pocket is through a small opening formed by the divergence of helices α6, α7, α8, and α8′. The stoichiometry of one drug molecule for two QacR subunits led to this asymmetric induction process, in which the drug-bound monomer undergoes a major structural change. Comparison of the drug-bound structure with the DNA-bound structure reveals that drug binding triggers a coil-to-helix transition of residues 89 to 93, which extends helix α5 by a turn. This transition removes the drug surrogates Tyr92 and Tyr93 from the hydrophobic core of the protein. Expulsion of these tyrosines also leads to the relocation of nearby helix α6 and its

tethered DNA-binding domain. The result of this structural transition is a 9-Å translation and a 37° rotation of the DNA-binding domain, effectively rendering the QacR dimer unable to bind its target DNA.

Three-Dimensional Structure of CprB

The gram-positive bacterial genus *Streptomyces* uses γ-butyrolactones as autoregulators or microbial hormones, together with their specific receptors (γ-butyrolactone receptors), to control morphological differentiation, antibiotic production, or both (150, 151). The most representative of the γ-butyrolactone autoregulatory factors is 2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone, known as A-factor, which is essential for aerial mycelium formation, streptomycin production, streptomycin resistance, and yellow pigment production (133, 134, 155) in *Streptomyces griseus*. However, the A-factor receptor protein, known as ArpA, has proved to be difficult to purify. In contrast, the CprB protein from *Streptomyces coelicolor* A3(2), which is 30% identical to ArpA (284), has been purified and crystallized (264), although the ligand for CprB is still unknown. Nonetheless, CprB binds the same nucleotide sequence as does ArpA (375) and indeed CprB also serves as a negative regulator for both secondary metabolism and morphogenesis in *S. coelicolor*, as ArpA does in *S. griseus* (264, 284).

The CprB dimer is omega shaped, and the two subunits in the dimer are related by a pseudo-twofold axis. Each monomer of CprB is composed of 10 α-helices and has two domains: a DNA-binding domain (residues 1 to 52) and a regulatory domain (residues 77 to 215). The three-dimensional structure of CprB is essentially similar to that of QacR bound to DNA except for the lack of α10 (350, 351). In addition, the DNA-binding domains of the two proteins are very similar, so much so that the two DNA-binding domains can be superimposed with an rms deviation of 1.48 Å for 71 Cα atoms (264). Although no information on CprB-operator DNA is available, the high degree of sequence conservation allowed the authors to predict that the core of the DNA-binding domain is composed of Ile14, Ile15, Ala18, Phe22, Leu32, Ile35, Leu46, and Phe50.

It has been suggested that a CprB dimer binds to its target DNA as found in the TetR–DNA complex (150, 287, 288). This is because structure-based amino acid sequence alignment shows that at the amino acid sequence level the DNA-binding domains of CprB and TetR are highly identical. This suggests that there is an evolutionary relationship between the DNA-binding domains of the two proteins. The regulatory domain of CprB is composed of six α-helices (helices α5 to α10) (264), which can also be superimposed on the corresponding domain of TetR (286, 287, 289) (PDB code 1JT0).

EthR Structure

Ethionamide has been used for more than 30 years as a second-line chemotherapeutic treatment in tuberculosis patients who have developed resistance to first-line drugs such as isoniazid and rifampin. Activation of the prodrug ethionamide is regulated by the Baeyer–Villiger monooxygenase EthA and the TetR family repressor EthR, whose open reading frames are separated by 75 bp in the genome of *Mycobacterium tuberculosis*. EthR has been shown to repress transcription of the activator *ethA* gene by binding to the intergenic region and contributing to ethionamide resistance.

The expression of *ethA* is regulated by EthR in *M. tuberculosis*. Overexpression of *ethR* leads to ethionamide resistance, whereas chromosomal inactivation of *ethR* promotes ethionamide hypersensitivity (28). EthR was found to bind directly and specifically to DNA sequences corresponding to the *ethRA* intergenic region (28, 90). The large EthR operator, which comprises 55 bp in comparison with the 15-bp operators recognized by most other family members, is organized as a putative highly degenerated palindrome containing pairs of overlapping inverted and tandem repeat sequences (90). In the absence of DNA, EthR forms a homodimer in solution, and surface plasmon resonance measurements suggest that EthR octamerizes when bound to DNA (90).

The EthR monomer is an all-helical, two-domain molecule (79). The N-terminal domain comprises helices 1 to 3, with helices 2 and 3 forming the HTH DNA-binding motif seen in other TetR family protein structures. The larger C-terminal domain, which in QacR and TetR has been dubbed the drug-binding domain, consists of helices 4 to 9, and its function in EthR is unknown. The crystal structure revealed that the dimerization interface, a conserved structural feature among the TetR class of repressors, is primarily formed by helices 8 and 9 (288, 351).

One of the most striking features of the EthR structure is a narrow tunnel-like cavity formed by helices 4, 5, 7, and 8 that opens to the bottom of the molecule (79). The tunnel measures about 20 Å in length and is lined predominantly, albeit not exclusively, by aromatic residues, with helices 5 and 7 constituting the majority of side chains. The loop connecting helices 4 and 5 restricts the opening of the hydrophobic tunnel, and the electron density in this loop is only poorly defined, indicating a certain degree of structural flexibility in the loop. This cavity may serve as the binding site for an as yet unknown ligand.

Crystal structure of TetR family members with unknown functions. New genomic/proteomic approaches are leading to the crystallization of a number of proteins, many of which have no assigned function. The following proteins of the TetR family have been crystallized: Cgl2612 of *Corynebacterium glutamicum* (pdb 1V7B); YbiH of *Salmonella enterica* serovar Typhimurium (pdb 1T33); YcdC of *Escherichia coli* (pdb 1PB6); and YfiR and YsiA from *Bacillus subtilis* (pdb 1RKT and 1VIO, respectively).

DNA-BINDING PREDICTIONS BASED ON TetR AND QacR CRYSTAL STRUCTURES

There is a perfect overlap of the DNA binding domains of QacR, TetR, CprB, and EthR, and no gaps were found in the α-helices involved in contacts with DNA in the multialignment of the 2,353 members of the TetR family in this domain. Based on these findings, we hypothesized that residues at the same position in the multialignment of all family members may play equivalent roles. This prompted us to analyze each amino acid in the multialignment within the DNA binding domain.

TABLE 4. Amino acid frequency at each of the positions critical for operator recognition by TetR family members[a]

22		33		34		35		37		38		39		43	
AA	%	AA	%	AA	%	AA	%	AA	%	AA	%	AA	%	AA	%
V	21.95	K	29.26	G	37.79	T	34.30	Y	74.16	R	22.75	H	44.50	K	77.25
L	20.60	R	20.07	A	18.93	S	20.87	F	8.05	Y	16.44	Y	33.83	R	10.07
M	18.66	P	10.27	P	12.55	A	19.6	H	4.63	H	13.29	R	4.90	L	2.82
I	17.65	Q	6.04	S	8.39	L	5.84	L	3.09	N	8.39	F	3.56	I	1.88
T	13.22	V	5.30	R	6.71	N	5.30	T	2.48	K	6.11	A	2.15	M	1.88
F	2.15	A	4.56	T	5.10	G	4.30	S	1.95	W	5.84	N	1.95	V	1.81
H	1.74	T	4.30	Q	3.56	V	2.28	N	1.88	A	5.64	Q	1.88	T	0.94
A	1.61	L	4.23	M	2.35	M	2.08	R	1.21	L	3.56	E	1.48	G	0.94
Y	1.28	E	3.36	N	1.07	Q	1.34	Q	0.6	S	3.56	L	1.34	Q	0.74
S	0.54	I	2.89	K	1.01	Y	1.28	A	0.47	F	2.89	W	1.21	A	0.47
P	0.40	H	2.89	V	0.81	I	1.14	I	0.47	Q	2.55	S	0.94	H	0.34
N	0.13	S	2.01	D	0.47	P	0.54	G	0.27	T	2.35	T	0.74	F	0.27
E	0.07	N	1.61	L	0.47	R	0.40	M	0.27	E	1.68	V	0.54	P	0.20
		G	1.07	E	0.40	E	0.20	V	0.2	V	1.61	C	0.40	S	0.13
		D	1.01	F	0.27	H	0.20	K	0.13	G	1.41	K	0.27	Y	0.07
		Y	0.67	I	0.07	K	0.13	C	0.07	D	1.07	I	0.13	E	0.07
		M	0.27	H	0.07	D	0.13	P	0.07	I	0.54	D	0.07	N	0.07
		C	0.13			C	0.07			C	0.20	G	0.07		
		F	0.07							M	0.07				
										P	0.07				

[a] The amino acid (AA) frequency is expressed as a percentage and refers to the 2,353 TetR family members.

Relationship between Profile Positions and Structural Positioning

Analysis comparison of the cocrystal of QacR and TetR with their corresponding operators revealed that residues corresponding to positions 22, 33, 34, 35, 37, 38, 39, and 43 in the family multialignment are involved in interactions with target operator DNA (Fig. 1). We analyzed the occurrence of each amino acid at these positions in the multialignment of all members of the TetR family (Table 4).

We found two types of position, one in which the residue was highly conserved and another in which the residue was poorly conserved, if at all. Positions 37, 39, and 43 were well conserved, whereas at positions 22, 33, 34, 35, and 38 the profile aligned different residues.

Tyr42 in TetR and Tyr40 in QacR corresponded to position 37 in the profile sequence displayed in Fig. 1, where a Tyr residue appeared in 74.16% of the aligned proteins (Table 4). The next most highly represented residues in this position are also aromatic amino acids: phenylalanine (8%) and histidine (4%) (Table 4). Tyr-42 in TetR and Tyr40 in QacR appear at the center of α-helix 3 and contact a thymine located at the center of the palindrome forming the operator and also contact a phosphate one position towards the center of the palindrome (Fig. 3B and 6B).

The residue at position 39 of the profile in the multialignment corresponds to His44 in TetR and His42 in QacR. In the corresponding cocrystals, these residues established contacts with the phosphate backbone (Fig. 3B and Fig. 6B). In the multiple sequence alignment of all family members, either histidine or tyrosine appears at position 39. We are tempted to propose that this residue is critical for interactions with the phosphate backbone.

A lysine-DNA phosphate interaction is shared at residues Lys48 in TetR and Lys46 in QacR, which correspond to posi-

tion 43 in the multialignment and are located in the amino end of the α4 helix. A lysine residue is present in 77% of TetR proteins, and their interactions with DNA phosphates seem to be crucial to adjust the HTH domain to contact DNA (Fig. 3B and 6B). At position 22 of the profile (Thr27 in TetR and Thr25 in QacR), five residues are the most abundant (Val, Leu, Met, Ile, and Thr). Thr27 in TetR and Thr25 in QacR are involved in interactions with the phosphate backbone.

Thus, in the TetR family, the contacts established by the residue aligned at position 37 in α3 (tyrosine present in 74% of the cases) and 39 in α3 (His or Tyr present in 98% of the cases) and a residue at position 43 in α4 (Lys present in 77% of the cases) probably orient the HTH motif to interact with the DNA major groove and anchor the protein to the phosphate backbone.

Glycine at position 16, located at the end of α1, in the multialignment is highly conserved and is involved in changing the polypeptide direction in the TetR and QacR crystals to orient the HTH DNA binding domain properly.

Positions 33, 34, 35, and 38 of the profile align many different residues (Table 4). In TetR and QacR, the corresponding residues establish specific contacts with different DNA bases except Asn38 of QacR (position 35 in the multialignment), which contacts the phosphate backbone. Based on the high variability of these positions in the corresponding multiple alignment of the family, we are tempted to propose that these positions endow specificity to each protein so that it can recognize its operator through specific protein-DNA interactions.

SOME REGULATORS ARE PART OF COMPLEX REGULATORY CIRCUITS

Published data indicate that the specific function of 85 members of TetR family is known (Table 2). More infor-

mation about each TetR protein is available at http://www .bactregulators.org (235). We have clustered the functions regulated by TetR family members into 10 groups (Table 2). The most frequent function performed by TetR family proteins is the regulation of efflux pumps and transporters involved in antibiotic resistance and tolerance to toxic chemicals. We have also observed that TetR family members often regulate their own synthesis, this feedback control ensures the transcriptional repressor level within optimal concentration limits (31, 73, 231, 338, 392). In this simple regulatory scheme, synthesis of the repressor and of the regulated protein(s) is derepressed in the presence of an inducer molecule.

However, TetR family proteins also participate in other types of regulatory networks that underlie complex processes, such as homeostasis in metabolism (biosynthesis of amino acids, nucleotides, protoheme, and reserve material), synthesis of osmoprotectants, quorum sensing, drug resistance, virulence, and processes related to growth phase-dependent differentiation (sporulation and biosynthesis of antibiotics) (Table 2) (www.bactregulators.org) (235).

Figure 8 shows a series of schemes in which a TetR family member plays a role in complex circuits. Below, for the sake of brevity, we have analyzed only some representative sets of regulatory networks, including proteins involved in drug resistance (AcrR of *E. coli* and MtrR of *Neisseria gonorrhoeae*), biosynthesis of an osmoprotectant (BetI), a key protein involved in idiophase antibiotic production and differentiation in *Streptomyces* (ArpR), a protein involved in pathogenesis in *Vibrio* (HapR), and some proteins involved in quorum sensing.

AcrR Regulator Is the Local Specific Regulator of the *acrAB* Efflux Pump

Multiple antibiotic resistance in *Escherichia coli* has attracted recent attention, promoting the elucidation of a number of mechanisms that contribute to this phenomenon. One of these is the transport of diverse substrates out of the cell by the AcrAB-TolC efflux transporter, leading to a multiple antibiotic resistance (Mar) phenotype (267). The set of antibiotics to which AcrAB can confer resistance includes ampicillin, chloramphenicol, erythromycin, fluoroquinolones, β-lactams, novobiocin, tetracycline, tigecycline, and rifampin (151, 187, 223, 267, 268, 276).

AcrB is a large cytoplasmic membrane protein (224, 226, 445, 446) which associates with AcrA, a membrane fusion protein (281), and TolC, a protein that forms a channel for the extrusion of substrates into the medium (102, 193). The *acrA* and *acrB* genes form an operon (224) whose transcription is regulated by the *acrR* gene product. The *acrR* gene is divergently transcribed from the *acrAB* operon. Overexpression of AcrR represses the transcription of *acrAB*. This observation is consistent with the function of AcrR as a repressor for *acrAB* transcription. Evidence for this function has come also from gel shift mobility assays, which provided direct evidence for the binding of AcrR to the promoter region of *acrAB*. DNA sequencing (92) of certain isolates that overexpressed *acrB* mRNA revealed that the mutant strains had insertions that disrupted the *acrR* gene or point mutations that rendered a nonfunctional regulator, i.e., an amino acid substitution of cysteine for arginine at position 45 of AcrR. This biochemical

and genetic evidence provides support for the regulatory role of AcrR.

MarA, SoxS, and Rob are related transcriptional activators of the AraC/XylS family (7, 112, 367) that activate *acrAB* expression, although they are not involved in the regulation of *acrAB* in response to general stress conditions (13, 14, 21, 35, 110, 224) because the *acrAB* operon can be activated in response to these stresses in genetic backgrounds lacking *mar* and *sox* (223–225). It was also found that general stress conditions increased the transcription of *acrAB* in the absence of functional AcrR, and these conditions, surprisingly, increased the transcription of *acrR* to a greater extent than that of *acrAB*. These results suggest the existence of a *mar-sox*-independent pathway to control *acrAB* expression in response to the general stress conditions. This transcriptional control of *acrAB* is also AcrR independent. Therefore, a major role of AcrR is to function as a specific secondary modulator to fine-tune the level of *acrAB* transcription and prevent unwanted overexpression of the efflux pump. This represents a novel mechanism for regulating gene expression in *E. coli*.

Mtr Circuit of *Neisseria*

The MtrCDE efflux pump of *Neisseria gonorrhoeae* provides gonococci with a mechanism to resist structurally diverse antimicrobial hydrophobic agents and antibiotic peptides that adopt β–sheet (protegenin 1) or two-helix (PC-8 and LC37) structures (130, 228, 238, 353). Mutations that render no expression or inactivation of *mtrR*, encoding a transcriptional repressor, resulted in high expression of the *mtrCDE* operon, concomitantly increasing resistance to hydrophobic agents (69, 130, 220, 221, 297, 353, 447). It was also found that strains of *N. gonorrhoeae* that display hypersusceptibility to hydrophobic agents often contained mutations in the *mtrCDE* efflux pump genes (406).

The *mtrR* gene is divergently transcribed with respect to the *mtrCDE* operon (Fig. 8F). The promoters of *mtrR* and *mtrC* overlap in their −35 boxes, and footprinting analysis showed that MtrR binds a 40-bp region within the −10 to −35 region of the *mtrR* promoter, which contains an inverted repeat (221). MtrR bound to its target site prevented expression from the efflux pump operon and its regulator (Fig. 8F). The expression of *mtr* genes is enhanced by the AraC/XylS member MtrA, although the mechanism of activation of this protein is unknown.

On the other hand, Veal and Shafer (407) have recently identified a gene that was designated *mtrF*, located downstream of the *mtrR* gene, that is predicted to encode a 56.1-kDa cytoplasmic membrane protein containing 12 transmembrane domains. Expression of *mtrF* was enhanced in a strain deficient in MtrR production, indicating that this gene, together with the closely linked *mtrCDE* operon, is subject to MtrR-dependent transcriptional control. Genetic evidence suggests that MtrF is also important in the expression of high-level detergent resistance by gonococci, and it was proposed that MtrF acts in conjunction with the MtrC–MtrD–MtrE efflux pump to confer high-level resistance to certain hydrophobic agents in gonococci. MtrR also controls the *farAB* operon, which encodes an efflux pump involved in resistance to long-chain fatty
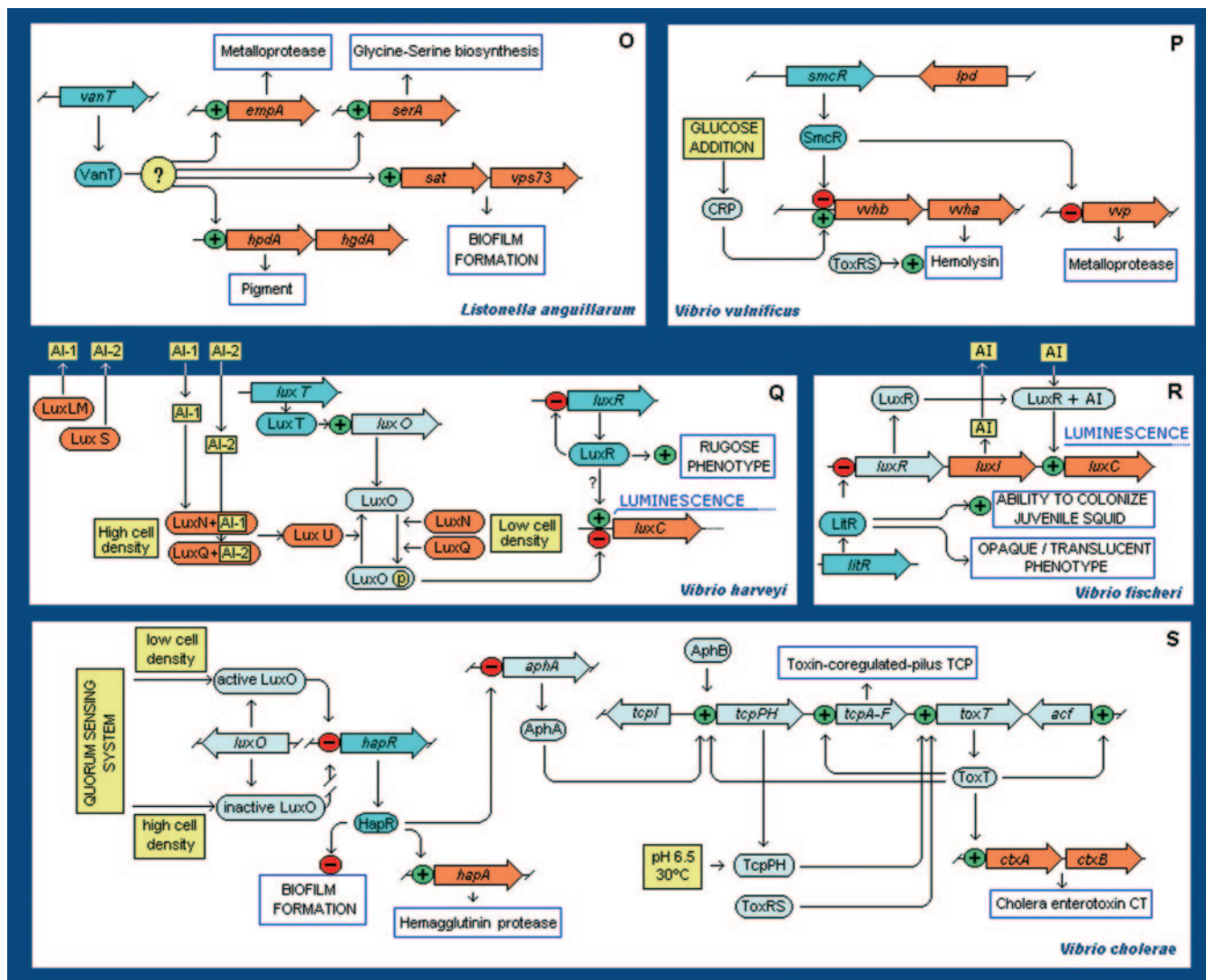
344
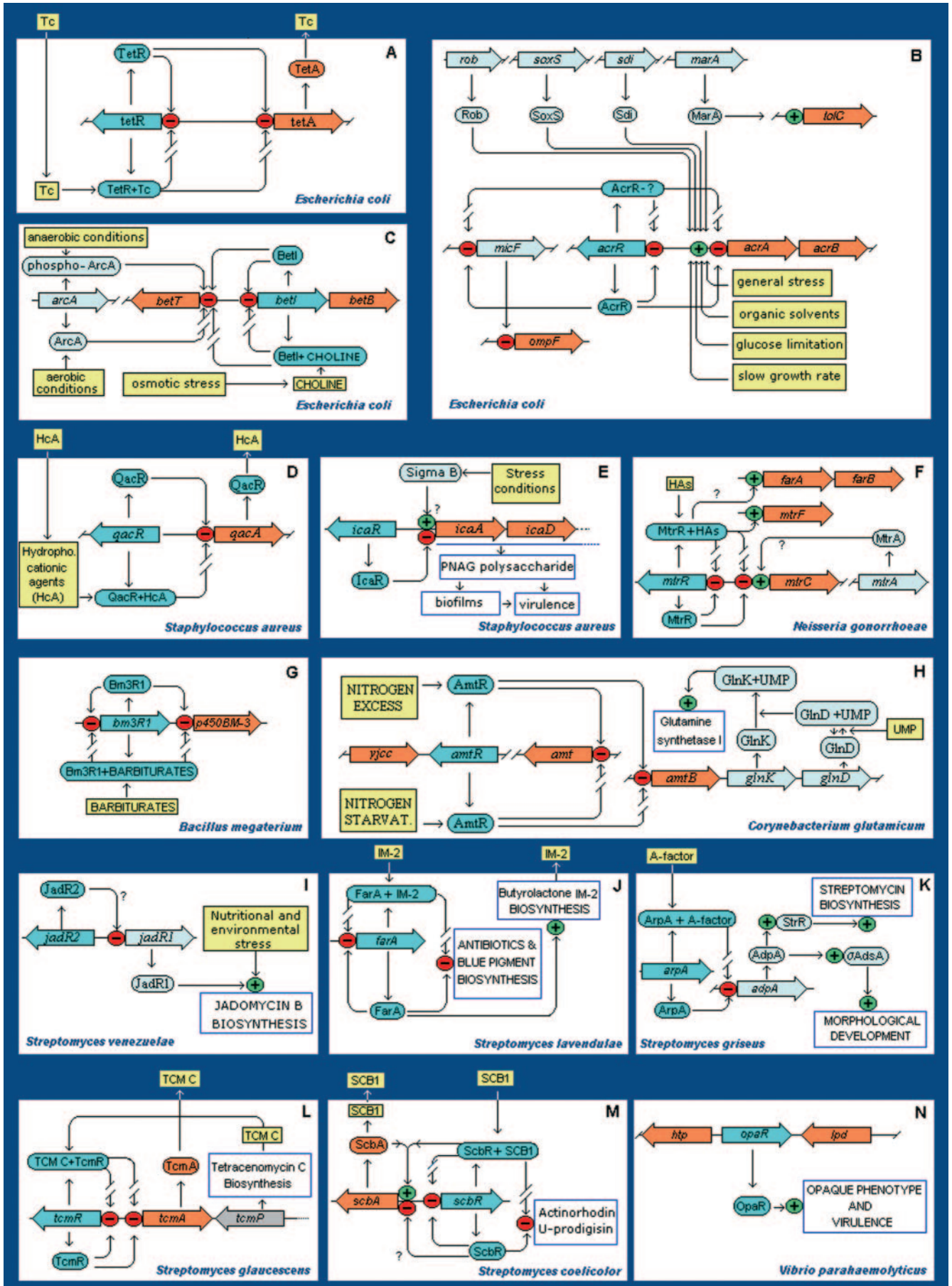
FIG. 8. Regulatory networks involving members of the TetR family. Although TetR and QacR cannot be considered part of a network, their type of control is shown because it is frequently found in members of the family. The following color code was used for complex networks: dark blue, TetR family member; orange, the gene directly regulated by the TetR family member; light blue, a regulator that modulates the expression of a TetR family member or which assists in the regulation of the gene under the control of a TetR family member; yellow boxes, signals and conditions influencing the system; open boxes, final results of the action of the system when the result is a scorable phenotype. References recommended for each circuit: panel A (29, 38, 286, 288, 388, 417, 418); panel B (4, 7, 13, 31, 35, 110, 176, 191, 230, 245, 270, 436); panel C (8, 91, 201–204, 222, 290, 331); panel D (119, 120, 122, 249, 261, 332, 350–351); panel E (5, 6, 66, 67, 116, 163); panel F (228, 238, 397, 333, 353, 408); panel G (87–89, 125, 140, 214, 215, 295, 360, 403); panel H (48, 161); panel I (78); panel J (56, 184, 185, 208, 413); panel K (133, 134, 158, 413); panel L (127); panel M (61, 266); panel N (240, 241, 345); panel O (248); panel P (59, 63, 242, 243, 355); panel Q (23, 24, 51, 107, 232, 255); panel R (97, 107, 117, 252, 341, 363); and panel S (107, 117, 181, 196, 217, 247).

acids (Fig. 8F). The efflux pump FarAB uses MtrE as the outer membrane component (208).

BetI Controls the Choline-Glycine Betaine Pathway of *E. coli*

In *Escherichia coli*, glycine betaine serves as an osmoprotector in hyperosmotically stressed cells. This osmoprotector accumulates in large amounts in the cytoplasm, which allows cells to maintain appropriate osmotic strength and thus prevents dehydration. Glycine betaine is only one of several cellular osmolytes used by *E. coli*, but its accumulation allows this

microbe to achieve its highest level of osmotic tolerance (199, 373). To accumulate glycine betaine, *E. coli* needs an external supply of this compound or its precursors choline and betaine aldehyde.

The osmoregulatory choline-glycine betaine pathway is encoded by the *bet* genes. The *betA* gene encodes choline dehydrogenase; *betB* encodes betaine aldehyde dehydrogenase; *betT* encodes a transport system for choline; and *betI* encodes a 21.8-kDa repressor protein involved in choline regulation of the *bet* genes (Fig. 8C). The *bet* genes are linked, with *betT* being transcribed divergently from the *betIBA* operon (203, 374). Primer extension analysis identified two partially over-

lapping promoters which were responsible for the divergent expression of the *betT* gene and *betIBA* operon. The transcripts are initiated 61 bp apart and are induced by osmotic stress, but for full expression choline is required in the growth medium (91, 202, 204). Because the ArcA protein represses the expression of *bet* genes in *E. coli* under anaerobiosis, the *bet* genes are expressed only under aerobic conditions. An *arcA* mutation caused complete derepression of the *bet* genes. A similar pattern for derepression by ArcA has been reported previously for other genes (*sodA* and *arcA*) which are directly regulated by ArcA (65).

Results from different laboratories suggest that choline regulation but not osmotic regulation of the *bet* promoters depended on BetI, a TetR family member. This was indicated by the requirement for choline, in addition to osmotic stress, for *betT* to be expressed in a mutant strain in which *betI* was supplied in *trans*. Furthermore, this choline effect was not seen in cells lacking *betI*. These findings indicate that *betI* encodes a repressor that reduced the expression of *betT* (331).

A chimeric BetI glutathione *S*-transferase fusion protein (BetI*) was purified, and gel mobility shift assays showed that BetI* formed a complex with a 41-bp DNA fragment carrying the intergenic *betI* promoter region. Footprinting revealed the presence of two sequences of dyad symmetry which probably constitute the BetI operator.

The *Sinorhizobium meliloti bet* genes have been cloned, and their involvement in response to osmotic stress has been analyzed (304, 305, 368).

ArpA Regulator from *Streptomyces*

Streptomycetes are filamentous, soil-living, gram-positive bacteria characterized for their ability to produce a wide variety of secondary metabolites, including antibiotics and biologically active substances, and for their complex morphological differentiation culminating in the formation of chains of spores (55). Secondary metabolite synthesis is sometimes called "physiological" differentiation because it occurs during the idiophase after the main period of rapid vegetative growth and assimilative metabolism (139, 153, 291).

The ultimate regulator of the mentioned processes in *Streptomyces griseus* is a homodimeric protein called A-factor receptor protein (ArpA) (155–158), which regulates the switch for physiological and morphological differentiation. The main biologically significant target of ArpA is the *adpA* gene. The AdpA protein in turn controls the expression of other genes. These genes include *strR*, which serves as a pathway-specific transcriptional activator for streptomycin biosynthetic genes (278); an open reading frame encoding a probable pathway-specific regulator for a polyketide compound (441); *adsA*, which encodes an extracytoplasmic function sigma factor of RNA polymerase essential for aerial mycelium formation (438); *sgmA*, which encodes a metalloendopeptidase probably involved in apoptosis of substrate hyphae during aerial mycelium development (174); *ssgA*, which encodes a small acidic protein essential for spore septum formation (437); *amfR*, essential for aerial hyphae formation (398, 440); and the *sprT* and *sprU* genes, which encode trypsin-like proteases (173).

In vitro, ArpA binds its target DNA site at the −10/−35 region, which is a 22-bp palindrome (5′-GG(T/C)CGGT(A/T)

(T/C)G(T/G)-3′). Addition of γ-butyrolactone effector to the ArpA-DNA complex immediately releases ArpA from the DNA. A mutant strain deficient in ArpA or producing a mutant ArpA protein unable to bind to its target DNA overproduces streptomycin and forms aerial mycelia and spores earlier than the wild-type strain (282, 283). An amino acid replacement at Val-41 to Ala in ArpA in the HTH motif at the N-terminal portion of ArpA abolished DNA binding activity but not γ-butyrolactone binding activity, suggesting the involvement of this HTH in DNA binding. On the other hand, mutation of Trp-119 (Trp 119→Ala) generated a mutant unable to bind the γ-butyrolactone, resulting in a mutant protein that did not sense the presence of A-factor. These data suggest that ArpA consists of an HTH DNA-binding at the N-terminal end and an effector binding domain at the C-terminal end of the protein.

In the streptomycin biosynthetic gene cluster in *S. griseus*, StrR is the pathway-specific regulator that serves as a transcriptional activator for the other genes in the cluster (320). Expression of the *strR* gene was controlled by the AdpA protein, which binds the region upstream of the *strR* promoter and activates its transcription (311, 312). *adpA* knockout mutants produced no streptomycin, and overexpression of *adpA* caused the wild-type *S. griseus* strain to produce streptomycin at an earlier growth stage in a larger amount. This set of events explains how A-factor triggers streptomycin biosynthesis.

Disruption of the chromosomal *adsA* gene encoding σ^AdsA resulted in loss of aerial hypha formation but not streptomycin production, indicating that this sigma factor is involved in morphological development (401, 438).

Several receptor proteins for γ-butyrolactone-type autoregulators have been described in other species of *Streptomyces*. For example, CprA and CprB are involved in secondary metabolism and aerial mycelium formation in *S. coelicolor* A3(2) (284). The virginiae butanolyde receptor BarA is involved in virginiamycin biosynthesis in *Streptomyces virginiae* (280), and the IM-2 receptor, FarA is involved in blue pigment production in another *Streptomyces* strain (413).

FarA in *Streptomyces lavendulae* senses the concentration of γ-butyrolactone IM-2 and also transduces this signal, thus derepressing antibiotic and blue pigment biosynthesis. In addition, FarA also seems to be necessary for IM-2 biosynthesis (Fig. 8J).

The role of ScbR protein in the quorum-sensing circuit of *Streptomyces coelicolor* is similar to that of FarA in *S. lavendulae*. ScbR is also involved in three functions: positively regulating SCB1 synthesis (the γ-butyrolactone that acts as signal), receiving the signal, and transducing this signal, thereby derepressing the production of the antibiotics actinorhodin and U-prodigiosin (Fig. 8M).

HapR Regulates Virulence Genes in *Vibrio cholerae*

The development of cholera in humans is directly related to the production of two virulence factors: toxin-coregulated pilus (TCP), which mediates colonization, and cholera toxin (CT), responsible for the severe diarrhea, characteristic of this disease. The coordinated expression of TCP and CT occurs though a complex regulatory cascade (Fig. 8S) in which the regulators AphA and AphB synergistically activate *tcpPH* tran-

scription (194). The TetR family protein HapR negatively regulates the expression of AphA and indirectly diminishes the production of TCP and CT.

HapR, in turn, is regulated by quorum-sensing signals that are sensed and transmitted by LuxO. The quorum-sensing apparatus in *Vibrio cholerae* is unusually complex and is composed of three parallel signaling systems (247). In contrast to other bacteria, in which high cell density triggers virulence gene expression, in *Vibrio cholerae* low cell density is the condition that activates the production of the pathogenic factors CT and TCP (455). At high cell density HapR positively regulates the expression of a hemagglutinin protease (Hap) that promotes detachment of *Vibrio cholerae* from the gastrointestinal epithelium (365) and exerts a negative effect on biofilm formation. Taking into account the regulatory functions of HapR and considering that some pathogenic biotypes lose HapR expression whereas others lose the *aphA* binding site, it appears that HapR expression is related to diminished toxicity and colonization capacity. These features offer potentially fruitful avenues of research to design drugs to modulate *Vibrio cholerae* pathogenicity.

Other Quorum-Sensing Circuits

Within the genus *Vibrio*, some TetR family proteins, i.e., LuxT, LuxR, and LitR, are involved in complex quorum-sensing circuits. In *Vibrio harveyi*, LuxT and LuxR participate in the circuit that regulates luminescence. This strain senses two autoinducers, AI-1 and AI-2 (58, 216). AI-1 is an acyl-homoserine lactone, as in other gram-negative bacteria. However, AI-2 is a novel autoinducer produced by many species that appears to be related to interspecies cell density detection. These two autoinducers use the same phosphorelay system to transduce the signal for bioluminescence regulation. Expression of *luxR* is in turn regulated by LitR (Fig. 8R).

BIOTECHNOLOGICAL APPLICATIONS AND FUTURE PROSPECTS

The Tet system is at present the most widely used system for conditional gene expression in eukaryotic cells (37). The system is based in the high affinity (10^{-9} M) of TetR for its operator, *tetO*, the favorable pharmacokinetics of tetracyclines (they diffuse through biological membranes), and their long record of safe clinical use. Cloning of the *tetO* elements adjacent to the TATA box of the target gene (114) was used successfully to control genes expressed by RNA polymerase I in *Leishmania donovani* and by RNA polymerase III in yeast and plant cells (76, 400). However, this system is not efficient in mammalian cells. For this reason, and based on the knowledge acquired about how TetR binds to its target operator, several chimeric versions of TetR fused to eukaryotic regulatory domains were constructed, such as the acidic activation domain (tTA) (22, 115, 403) and repression domains (tTS) (33, 336). Based on hybrid transregulators, transgenic mice able to produce diphtheria toxin or the regulated expression of Shiga toxin β to induce apoptosis in mammalian fibroblastic cells were obtained.

The *tet* system has also been used to study cancer and neurological disorders (95, 98, 419). In the future, advances to

approach multifactorial biological processes like development and diseases are expected, which will be relevant for the treatment of complex diseases (37).

Solvent efflux pumps generally exhibit a broad substrate specificity, but some of them are highly specific and remove a certain number of chemicals. One such efflux pumps is the TtgABC pump of *Pseudomonas putida* DOT-T1E, which removes toluene, *m*-xylene, and propylbenzene as well as styrene and other aromatic hydrocarbons (327) in addition to several antibiotics. This efflux pump is under the control of the drug binding repressor TtgR, a member of the TetR family (391), and has applications in the safe development of solvent-resistant bacteria. Therefore, it is potentially suitable for the biotransformation of water-insoluble compounds into added-value products. One such system has been exploited to produce catechols from xylenes/toluenes in double-phase systems (328). Because these efflux systems are energy consuming, their expression in heterologous hosts has to be tightly controlled by their cognate repressor, which in turn has to be able to respond to the presence of the aromatic solvent. These types of pumps are presumed to be useful if transferred to a wide variety of bacteria that carry suitable biotransformation machineries.

As shown in Fig. 8, TetR family members are key players in multidrug resistance, virulence, and pathogenicity processes in certain bacterial pathogens. The development of drugs that bind irreversibly to the repressors and prevent their release from their cognate operators may be a strategy to fight these pathogens. Suitable screening procedures to search for these drugs can be envisaged based on current tools for gram-negative bacteria (74).

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