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Review

Sensing and responding to diverse extracellular signals? Analysis of the sensor kinases and response regulators of *Streptomyces coelicolor* A3(2)

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Streptomyces coelicolor is a Gram-positive soil bacterium that undergoes a complex developmental life cycle. The genome sequence of this organism was recently completed and has revealed the presence of over 60 sigma factors and a multitude of other transcriptional regulators, with a significant number of these being putative two-component signal transduction proteins. The authors have used the criteria established by Hoch and co-workers (Fabret *et al*., 1999, *J Bacteriol* 181, 1975–1983) to identify sensor kinase and response regulator genes encoded within the *S. coelicolor* genome. This analysis has revealed the presence of 84 sensor kinase genes, 67 of which lie adjacent to genes encoding response regulators. This strongly suggests that these paired genes encode two-component systems. In addition there are 13 orphan response regulators encoded in the genome, several of which have already been characterized and are implicated in development and antibiotic production, and 17 unpaired and as yet uncharacterized sensor kinases. This article attempts to infer useful information from sequence analysis and reviews what is currently known about the two-component systems, unpaired sensor kinases and orphan response regulators of *S. coelicolor* from both published reports and the authors' own unpublished data.

Two-component signal transduction systems (TCSs), consisting of a sensor kinase (SK) and a cognate response regulator (RR), are found across all three domains of life, the Bacteria, Archaea and Eukarya. They are most widespread in the Bacteria (with the exception of the mycoplasmas) but SK genes have also been identified in the Archaea (Kim & Forst, 2001), in fungi and protozoa (Thomason & Kay, 2000), and in plants (Hwang *et al*., 2002). SK genes are conspicuously absent from the animal genomes so far sequenced and it has been proposed that these proteins are not present in the animal kingdom as a whole (Wolanin *et al*., 2002). This potentially makes them an attractive target for antimicrobials (Barrett *et al*., 1998), especially since some bacteria, including *Bacillus subtilis* and the opportunistic pathogen *Staphylococcus aureus*, contain essential TCSs (Fabret & Hoch, 1998; Martin *et al*., 1999). The extracytoplasmic sensor domain of each SK responds to specific types of environmental stimuli. The signal is transferred via autophosphorylation of a conserved His residue in the cytoplasmic H box to the aspartate residue of the cognate RR, which then activates transcription of target genes (Hakenbeck & Stock, 1996; Fig. 1). In bacteria, generally speaking the range of environmental stimuli to which an organism can respond is directly linked to the number of SKs encoded by that organism's genome. The number of SKs encoded by a bacterial genome is also proportional to the size of the genome, such that in bacteria which are obligate pathogens, and generally have smaller genomes, the percentage of SK and RR pairs in relation to the total number of genes is quite small, approximately 0·26 % compared to 0·65 % in free-living bacteria (Kim & Forst, 2001). *Pseudomonas aeruginosa*, however, has a large number of regulatory genes including 118 encoding RRs (Stover *et al*., 2000), and this may be a defining trait of opportunistic pathogens.

Streptomyces coelicolor is a high-G+C, Gram-positive bacterium that exhibits a complex developmental life cycle. Spore germination and subsequent outgrowth leads to a network of vegetative hyphae. In response to any of a number of proposed signals, including nutritional stress and an extracellular signalling cascade, the substrate

The online version of this review (at http://mic.sgmjournals.org) contains supplementary figures showing results from the alignment of residues surrounding conserved histidine residue in SKs, and from TopPred 2 analysis predicting the membrane topology of all 84 SK sequences.

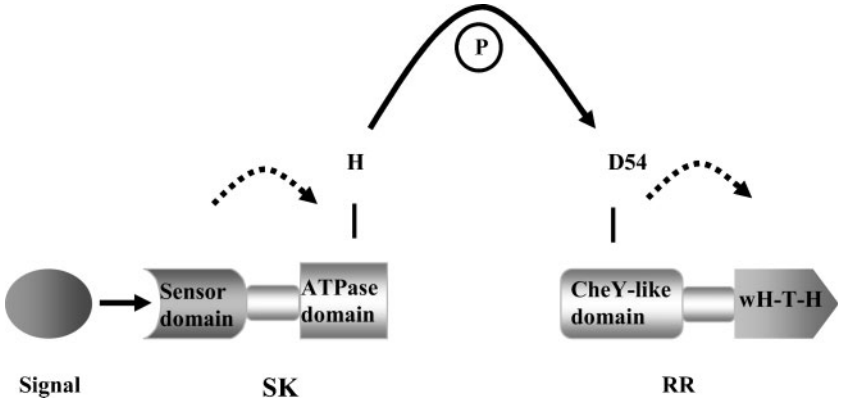

Fig. 1. Schematic representation of the basic two-component signalling pathway.

mycelium gives rise to aerial hyphae that eventually become septate to form mature spores. Since *S. coelicolor* is a soil-dwelling organism it needs to respond to highly variable conditions within its environment.

The complete genome sequence of *S. coelicolor* (Bentley *et al.*, 2002) has allowed us to analyse the TCSs, unpaired SKs and orphan RRs of this organism. Although these systems are widespread throughout the bacteria, very little is known about the signals sensed by the SK or, in many cases, the targets for the RR of each system. By searching the *S. coelicolor* genome for proteins containing both the SK ATPase domain and the conserved histidine motif (Fabret *et al.*, 1999) we found 84 SK genes. Searching for the RR effector domain together with the CheY-like domain for interaction with SKs revealed 80 RR genes. This differs slightly from the figures (85 SKs and 79 RRs) reported in the genome sequence paper (Bentley *et al.*, 2002), probably because the hybrid SCO4009 is listed as an SK gene in the sequence annotation whereas our analysis suggests it is not a true SK, since it lacks the conserved histidine for autophosphorylation. Of these 84 SK genes, 67 are adjacent to RR genes, while 17 are unpaired.

As well as the 67 paired RR genes there are 13 orphan RR genes. The paired SK and RR genes account for approximately 0·86 % of the total ORFs in *S. coelicolor*, which is 25 % higher than the average for non-pathogenic, free-living bacteria (Kim & Forst, 2001). This, as pointed out by Bentley *et al.* (2002), suggests that this organism might be well equipped to deal with a wide range of environmental stimuli.

Initial identification and classification of SKs and RRs

Taking as a starting point the analysis of Hoch and co-workers (Fabret *et al.*, 1999) we set about classifying the 84 SK genes and 80 RR genes of *S. coelicolor*. The SKs were identified by searching the genome sequence for proteins containing SCOP domain 55874 (ATPase domain; Murzin *et al.*, 1995) using hidden Markov models (HMMs) for this domain obtained from the Superfamily database (Gough *et al.*, 2001) and the hmmsearch program of the HMMER package version 2.2 (2001; S. Eddy, http://hmmer.wustl.edu). To search for the conserved histidine, the site of autophosphorylation, alignments of the 16 amino acid residues surrounding the conserved histidine for each of the groups I, II IIIa, IIIb and IV were taken from Fabret *et al.* (1999) and used to make HMMs using the hmmbuild program of the HMMER package. The five HMMs thus made were used to search each of the 84 SKs of *S. coelicolor* using the program hmmpfam of the HMMER suite (see supplementary figure S1 with the online version of this paper at http://mic.sgmjournals.org). The scores of the proteins with these HMMs were used to assign them to different groups. In each case it was verified that the His alignment did indeed identify the conserved histidine in the *S. coelicolor* sequence. As pointed out by Fabret *et al.* (1999), alignment of this region is more informative than alignment of the whole protein sequence since the sensor domains of SKs differ greatly and are likely to skew the analysis. Alignment of these sequences revealed that the SKs of *S. coelicolor* fell into the five main groups, I, II, IIIa, IIIb and IV, as defined in *B. subtilis* (Fabret *et al.*, 1999), where groups IIIa and IIIb are very closely related. As in *B. subtilis*, the vast majority fell into groups II and IIIa, with only one falling into group I, two into group IIIb and five into group IV (Table 1). It was notable that, as in *B. subtilis*, all of the group II SKs were paired with (i.e. adjacent to) RRs belonging to the NarL family, while all but two of the paired group IIIa SKs were linked with RRs belonging to the OmpR family. The remaining two paired group IIIa SKs (SCO0871 and SCO5748) are paired with RRs that lack a DNA binding domain, and three group IIIa SKs are unpaired. Three of the group IV SKs (SCO1136, SCO5434 and SCO5828) are paired with RRs containing atypical winged helix–turn–helix domains, while the remaining two group IV SKs and the only two group IIIb SKs are unpaired (Table 1). Similar to SKs, the RRs were identified by searching the genome for proteins containing SCOP

Table 1. Classification and novel domains of the SKs

Abbreviations: NADP, NADP binding domain; DNA, DNA binding domain; PAS, PAS domain; cAMP, cyclic AMP binding domain; GAF, GAF domain; ABP, adenine nucleotide binding protein. The TM domains column shows the positions of the most likely transmembrane helices as predicted by TopPred 2 analysis. For the RRs: *Lacks CheY-like domain for SK interaction; DBD, no DNA binding domain; wHtH, winged helix–turn–helix. HR or RH refers to the gene order of the RR and SK genes.

SK	Group	Domains	TM domains	Sensor domain length (aa)	RR	Group	Gene order	Function
SCO1217	I	None	7–27, 201–221	174	Unpaired	–	–	Unknown
AbsA1	II	None	70–90, 115–135	25	AbsA2	NarL	HR	Secondary metabolism
ChiS	II	None	13–33, 137–157	104	ChiR	NarL	HR	Chitinase production
SCO0203	II	GAF	239–259	?	SCO0204	NarL	RH	Growth dependent
SCO0211	II	None	None	None	Unpaired	–	–	Unknown
SCO0422	II	None	74–94, 104–124	10	SCO0421	NarL	HR	Unknown
SCO1071	II	None	37–57, 106–126	49	SCO1070	NarL	HR	Unknown
SCO1259	II	None	97–117, 139–159	32	SCO1260	NarL	HR	Unknown
SCO1369	II	None	40–60, 71–91	11	SCO1370	NarL	HR	Unknown
SCO1744	II	None	48–68, 119–139	51	SCO1745	NarL	HR	Unknown
SCO1802	II	GAF	None	None	SCO1801	NarL	HR	Unknown
SCO2121	II	None	45–65, 103–123	38	SCO2120	NarL	HR	Unknown
SCO2166	II	NADP	17–37, 120–140	83	SCO2165	NarL	HR	Unknown
SCO2215	II	None	28–48, 175–195	127	SCO2216	NarL	HR	Unknown
SCO2307	II	None	43–63, 121–141	58	SCO2308	NarL	HR	Unknown
SCO2359	II	None	85–105, 121–141	16	SCO2358	NarL	HR	Unknown
SCO2452	II	GAF	169–189, 270–290	81	Unpaired	–	–	Unknown
SCO2518	II	None	61–81, 152–172	71	SCO2517	NarL	HR	Developmental
SCO3119	II	None	49–69, 110–130	41	Unpaired	–	–	Unknown
SCO3390	II	None	13–36, 41–61	5	SCO3389	NarL	HR	Unknown
SCO3639	II	None	95–115, 216–236	101	SCO3638	NarL	HR	Unknown
SCO3641	II	None	129–149, 180–200	31	SCO3640	NarL	HR	Unknown
SCO3654	II	None	63–83, 130–150	47	SCO3653	NarL	HR	Developmental
SCO3750	II	None	113–133, 164–184	31	Unpaired	–	–	Unknown
SCO3757	II	None	49–69, 150–170	81	SCO3756	NarL	HR	Unknown
SCO3948	II	GAF	224–244, 540–560	296	Unpaired	–	–	Unknown
SCO4073	II	None	48–68, 166–186	98	SCO4072	NarL	HR	Unknown
SCO4124	II	None	98–118, 125–145	7	SCO4123	NarL	HR	Unknown
SCO4275	II	None	53–73, 93–113	20	SCO4276	NarL	HR	Unknown
SCO4362	II	None	89–109, 133–153	24	SCO4363	NarL	HR	Unknown
SCO4597	II	None	61–81, 157–177	76	SCO4596	NarL	HR	Unknown
SCO4598	II	None	53–73, 139–159	66	Unpaired	–	–	Unknown
SCO4667	II	None	39–59, 179–199	120	SCO4668	NarL	HR	Unknown
SCO4791	II	None	57–77, 106–126	29	SCO4792	NarL	HR	Unknown
SCO5131	II	None	46–66, 141–161	75	SCO5132	NarL	HR	Unknown
SCO5454	II	None	108–128, 166–186	38	SCO5455	NarL	HR	Unknown
SCO5683	II	None	36–56, 144–164	88	SCO5684	NarL	HR	Unknown
SCO5784	II	None	44–64, 67–87	3	SCO5785	NarL	HR	Unknown
SCO5824	II	None	52–72, 99–119	27	SCO5825	NarL	HR	Unknown
SCO6139	II	None	90–110, 142–162	32	SCO6140	NarL	HR	Unknown
SCO6163	II	None	1–21, 24–44	3	SCO6162	NarL	HR	Unknown
SCO6253	II	None	110–130, 162–182	32	SCO6254	NarL	HR	Unknown
SCO6268	II	None	None	None	Unpaired	–	–	Unknown
SCO6362	II	None	98–118, 166–186	48	SCO6363	NarL	HR	Unknown
SCO6421	II	None	11–31, 98–118	67	SCO6422	NarL	HR	Unknown
SCO6424	II	None	21–41, 44–64	3	Unpaired	–	–	Unknown
SCO6668	II	None	25–45, 71–91	26	SCO6667	NarL	HR	Unknown
SCO6794, CvnA7	II	None	42–62, 75–95	13	Unpaired	–	–	Unknown

Table 1. cont.

SK	Group	Domains	TM domains	Sensor domain length (aa)	RR	Group	Gene order	Function
SCO7089	II	None	15–35, 111–131	76	SCO7088	NarL	HR	Unknown
SCO7649	II	None	80–100, 138–158	38	SCO7648	NarL	HR	Unknown
SCO7711	II	None	90–110, 402–422	292	SCO7712	NarL	HR	Unknown
CseC	IIIa	None	60–80, 183–203	103	CseB	OmpR	RH	Cell wall damage
AfsQ2	IIIa	None	32–52, 199–219	147	AfsQ1	OmpR	RH	Unknown
VanS	IIIa	None	17–37, 65–85	28	VanR	OmpR	RH	Vancomycin resistance
CutS	IIIa	None	38–58, 121–141	63	CutR	OmpR	RH	Unknown
PhoR	IIIa	None	6–26, 340–360	314	PhoP	OmpR	RH	Phosphate concentration
KdpD	IIIa	ABP	379–399, 456–476	57	KdpE	OmpR	RH	Osmotic shock
SCO0551	IIIa	None	7–27, 142–162	115	SCO0552	OmpR	RH	Unknown
SCO0871	IIIa	None	10–30, 187–207	157	SCO0870	DBD	RH	Unknown
SCO2142	IIIa	None	163–183	?	SCO2143	OmpR	HR	Unknown
SCO2800	IIIa	None	8–28, 149–169	121	SCO2801	OmpR	RH	Unknown
SCO3012	IIIa	None	76–96, 263–283	167	SCO3013	OmpR	RH	Unknown
SCO3062	IIIa	None	7–27, 125–145	98	SCO3063	OmpR	RH	Unknown
SCO3740	IIIa	None	43–63, 69–89	6	SCO3741	OmpR	RH	Unknown
SCO4021	IIIa	None	16–36, 185–205	149	SCO4020	OmpR	RH	Unknown
SCO4155	IIIa	None	20–40, 167–187	127	SCO4156	OmpR	RH	Unknown
SCO5282	IIIa	None	34–54, 61–81	7	SCO5283	OmpR	RH	Unknown
SCO5304	IIIa	None	16–36, 137–157	101	Unpaired	–	–	Unknown
SCO5404	IIIa	None	15–35, 183–203	148	SCO5403	OmpR	RH	Unknown
SCO5748, OsaA	IIIa	GAF	1146–1166, 1235–1255	69	SCO5749, OsaB	DBD	HR	Osmoadaptation
SCO5779	IIIa	None	5–25, 173–193	148	SCO5778	OmpR	RH	Unknown
SCO6353	IIIa	None	28–48, 169–189	121	SCO6354	OmpR*	RH	Unknown
SCO6369	IIIa	None	8–28, 140–160118	112	Unpaired	–	–	Unknown
SCO7076	IIIa	None	25–45, 163–183	118	SCO7075	OmpR	RH	Unknown
SCO7231	IIIa	None	17–37, 187–207	150	SCO7230	OmpR	RH	Unknown
SCO7327	IIIa	GAF, DNA	629–649	?	Unpaired	–	–	Unknown
SCO7534	IIIa	None	27–47, 197–217	150	SCO7533	OmpR	RH	Unknown
SCO7297	IIIb	cAMP	48–68	?	Unpaired	–	–	Unknown
SCO1596	IIIb	PAS	None	None	Unpaired	–	–	Developmental
SCO0676	IV	PAS, GAF	6–26, 163–183	137	Unpaired	–	–	Unknown
SCO1137	IV	PAS, NADP	15–35, 177–197	142	SCO1136	wHtH	HR	Unknown
SCO5239	IV	PAS	None	None	Unpaired	–	–	Unknown
SCO5435	IV	PAS	17–37, 172–192	135	SCO5434	wHtH	HR	Unknown
SCO5829	IV	PAS, NADP	36–56, 194–214	138	SCO5828	wHtH	HR	Unknown

domain 52172 (CheY-like domain for interaction with SK). This search identified 80 RRs, 75 of which contain SCOP domain 46894 (the C-terminal effector domain of the bipartite RRs, for DNA binding), three contain a different effector domain (SCOP 46785, winged helix–turn–helix domain), as mentioned above, and another two (SCO0870 and SCO5749) contain no recognized DNA binding domain (Table 1). A single gene (SCO4009) is annotated as a bifunctional protein (SK and RR) but, while it contains domains common to both, it does not appear to fit in either group. SCO4009 contains the ATPase domain common to all SKs, the homodimerization domain found in many SKs and the CheY-like receiver domain common to all RRs. However, it lacks both the conserved His residue for autophosphorylation and the C-terminal effector domain of the bipartite response regulators, which is required for binding to DNA.

Sensor domains

The N-terminal domains of SKs have very low sequence homology, probably due to the diverse range of signals sensed by these proteins. As such these domains were excluded from the analysis used to group the 84 SKs in the *S. coelicolor* genome. However, in order to learn more about the sensor domains, the sequence of each SK was analysed using TopPred II, a membrane topology prediction package (http://bioweb.pasteur.fr/seqanal/interfaces/toppred.html; Claros & von Heijne, 1994). Where available, the two best matches for each SK were assumed to be the

true transmembrane (TM) domains (Table 1). Surprisingly, five of the SKs are predicted to be soluble cytoplasmic proteins, four had only a single TM domain and over half are predicted to have three or more TM domains (see supplementary figure S2 with the online version of this paper at http://mic.sgmjournals.org). Of the five soluble SKs, four are unpaired (Table 1), and none of these contains the HPt domain (characteristic of proteins involved in signalling cascades). However, it is possible that these soluble kinases may be activated by another SK as in the case of *Escherichia coli* CheA (Levit *et al.*, 1996).

The sensor domain includes all the residues between the end of the first and the start of the second TM domain, and the size of the sensor domains ranged from 3 to 314 amino acids (Table 1). Ten SKs contain sensor domains of less than 20 amino acids, suggesting that they may belong to a new subfamily of intramembrane sensing SKs (Mascher *et al.*, 2003). SKs belonging to this recently discovered subfamily are predicted to sense changes in membrane structure or topology. Mascher and colleagues first identified SKs of this type in *B. subtilis* and then searched all the microbial genome sequences using a defined set of criteria. In order to fit into this subfamily the proteins must be less than 400 residues in length, with an N-terminal domain less than 100 amino acids in length (including both TM domains) and a sensor domain of less than 20 residues. They identified only four intramembrane sensing SKs in *S. coelicolor*, although our analysis shows that in fact five out of the ten SKs described here fit these criteria. One of these (SCO3740) therefore escaped their initial analysis (Mascher *et al.*, 2003). The remaining five either have N-terminal domains that are too long or the length of the whole protein exceeds 400 residues so that, despite exceeding the cut-off criteria, these proteins may still be intramembrane sensing SKs. The 20 residue cut-off point for these domains appears to be critical since there is strong evidence that *S. coelicolor* VanS, which has a sensor domain of only 27 residues, responds to vancomycin and related glycopeptides directly, presumably by binding of the drug to the sensor domain (Hong *et al.*, 2004).

GAF and PAS domains: new partners for the SK domains

The *S. coelicolor* genome encodes seven GAF-containing SKs (Table 1). GAF domains have been identified in many bacterial proteins, including adenylate cyclases, phosphotransferases and SKs, and appear to act as binding sites for small ligands which modulate the catalytic activity of the target protein (Aravind & Ponting, 1997). It seems likely therefore that the signals for these GAF-containing SKs are small ligands that induce autophosphorylation of the SK and subsequent signal transduction to activate or repress the expression of target genes.

The closely related PAS domain is also found in six of the *S. coelicolor* SKs (Table 1). Both PAS and GAF domains are implicated in signalling. However, while GAF domains bind ligands such as nucleotides and small molecules, PAS domains appear to bind flavins, haems and chromophores and are responsive to oxygen, redox potential and light (Zhulin *et al.*, 1997; Taylor & Zhulin, 1999). *S. coelicolor* synthesizes a yellow/orange pigment when exposed to light but produces colourless colonies when grown in the dark. This raises the intriguing possibility that *S. coelicolor* may modulate gene expression in response to light, which is probably sensed by one or more of these PAS-containing SKs and the signal transduced through their cognate RRs.

Functions and phenotypes of TCSs

The functions and targets of the 67 *S. coelicolor* TCSs are largely unknown, and those with assigned functions are usually homologous to TCSs from other organisms. However, phenotypic and/or microarray data are available for many of the TCSs of *S. coelicolor* and here we summarize what is known so far.

PhoPR. The *phoPR* operon of *B. subtilis* encodes a TCS that directly activates expression of Pho regulon genes under low-phosphate conditions. The Pho regulon includes operons encoding a high-affinity phosphate transport system, enzymes involved in synthesis of the cell wall polymer teichuronic acid (which replaces teichoic acid in phosphate-starved cells), enzymes involved in teichoic acid biosynthesis, two genes encoding the major alkaline phosphatases of *B. subtilis*, PhoA and PhoB, and the *phoPR* operon itself (Shi & Hulett, 1999). As with most TCSs the exact ligand bound by the SK, PhoR, has yet to be identified, but the signal for PhoPR activation of the Pho regulon appears to be phosphate starvation and results in an increase in the availability of phosphate in the cell.

In the actinomycete *Mycobacterium tuberculosis* the *phoPR* genes were identified on the basis of similarity to the *B. subtilis* genes (Cole *et al.*, 1998). In *M. tuberculosis*, a *phoP* mutant strain is attenuated *in vivo* in a mouse infection model, and it was proposed that these genes are more similar to the *phoPQ* genes that control virulence in *Salmonella* sp. than they are to the *phoPR* genes that control the Pho regulon of *B. subtilis* (Perez *et al.*, 2001). Alternatively, deletion of this TCS may result in down-regulation of the Pho-regulon genes, resulting in attenuation. In *S. coelicolor* and the closely related *Streptomyces lividans* the *phoPR* genes were also identified on the basis of similarity to the *B. subtilis* genes, but these genes were found to lie close to genes involved in phosphate transport, strengthening the case for their being true *phoPR* homologues (Sola-Landa *et al.*, 2003). The *S. lividans* genes were cloned and characterized, and phosphorylated PhoP was found to activate expression of *phoA* (alkaline phosphatase), apparently in response to low phosphate concentrations (Sola-Landa *et al.*, 2003). Phosphorylated PhoP is also implicated in the regulation of a high-affinity phosphate transporter, since there is a drastic reduction in phosphate uptake in a *phoPR* mutant strain (Sola-Landa *et al.*, 2003). The same authors

reported that production of actinorhodin and undecylprodigiosin was greatly increased in the *phoPR* background, suggesting that PhoP may negatively regulate production of these secondary metabolites.

CseBC. Cse denotes control of sigma E, where σ^E is an ECF (extra-cytoplasmic function) sigma factor. The *cse* genes are found in the *sigE* operon, which includes *cseA*, encoding a negative regulator of *sigE* expression (M. I. Hutchings, H. J. Hong, E. Leibowitz & M. J. Buttner, unpublished), *cseB*, encoding the RR, and *cseC*, encoding the SK. Expression of *sigE* is induced by a range of cell-wall-specific antibiotics, which inhibit late steps of peptidoglycan biosynthesis, and also by the cell wall hydrolytic enzyme lysozyme (Hong *et al.*, 2002). The *sigE* and *cseB* phenotypes are identical, i.e. hypersensitivity to lysozyme and a requirement for high concentrations of magnesium for normal growth, because *sigE* expression is absolutely dependent on CseB. This suggests a model in which CseC, sensing cell wall damage, is autophosphorylated at conserved histidine residue 271 and in turn phosphorylates CseB at Asp-55, which then activates expression of the *sigE* operon (Paget *et al.*, 1999). The *sigE* promoter is not transcribed by σ^E-containing RNA polymerase ($E\sigma^E$) holoenzyme (Paget *et al.*, 1999) and the only targets so far identified for $E\sigma^E$ are one of two promoters for *hrdD*, a sigma factor of unknown function, and the promoter of the *cwg* operon, which appears to encode enzymes involved in the synthesis of a cell wall glycan (Hong *et al.*, 2002). It seems likely from the evidence gathered so far that at least some of the targets for $E\sigma^E$ are involved in cell wall homeostasis.

VanRS. The SK VanS and its cognate RR VanR control inducible vancomycin resistance in enterococci and in glycopeptide-producing strains such as *Streptomyces toyacaensis* (Pootoolal *et al.*, 2002). Vancomycin is a cell-wall-specific antibiotic that binds to the D-Ala-D-Ala terminus of the stem peptide of Lipid II to prevent transpeptidation and, hence, to prevent cell wall assembly, resulting in osmotic lysis. Vancomycin-resistant strains reprogramme their cell walls so that the stem peptide ends D-Ala-D-Lac, a substrate for which vancomycin has 1000-fold lower specificity (Billot-Klein *et al.*, 1997). The enzymes that catalyse the reprogramming of the cell wall peptidoglycan are VanH, a D-lactate dehydrogenase, VanA, a D-Ala-D-Lac ligase, and VanX, a D-alanyl-D-alanine dipeptidase that cleaves stem peptides ending D-Ala-D-Ala.

A vancomycin resistance gene cluster was recently identified in *S. coelicolor*, containing seven genes: *vanRS*, *vanJ*, *vanK* and *vanHAX*. The *vanHAX* operon encodes the enzymes that reprogramme peptidoglycan biosynthesis, *vanJ* encodes a protein of unknown function that is not required for vancomycin resistance and *vanK* encodes a homologue of the Fem family of non-ribosomal peptide synthetases (Rohrer & Berger-Bachi, 2003). Deletion of *vanK* resulted in a drug-sensitive phenotype, suggesting that *S. coelicolor* has a novel mechanism of vancomycin resistance, since this gene has not been found in the vancomycin resistance clusters of any other bacteria (Hong *et al.*, 2004). The expression of the seven genes in this cluster, divided into four transcription units, is absolutely dependent on the VanRS TCS, and microarray analysis has revealed that these are the only genes in the VanR regulon (M. I. Hutchings & M. J. Buttner, unpublished). Interestingly, of the four transcripts encoding these *van* genes, all but the *vanJ* transcript are leaderless (Hong *et al.*, 2004), a situation that appears to be more common in the streptomycetes than in other bacteria (Janssen, 1993).

KdpDE. Annotated as a putative turgor pressure sensor (KdpD) and putative turgor pressure regulator (KdpE) based on primary sequence similarity to the *E. coli* turgor-sensing TCS proteins, the Kdp TCS has been most extensively studied in *E. coli* but appears to be ubiquitous amongst bacteria. The signal sensed by KdpD is still unclear, although autophosphorylation in a reconstituted *in vitro* system appears to require the presence of divalent Mg^{2+} or Ca^{2+} and monovalent Na^+ or K^+. It has, however, been widely proposed that KdpD senses turgor pressure directly and that this pressure results in conformational change, autophosphorylation and subsequent phosphotransfer (Jung & Altendorf, 2003). In *E. coli*, *kdpD* and *kdpE* are the last two genes in the *kdpFABCDE* operon, which, besides the TCS, encodes the potassium-transporting KdpFABC ATPase. Expression of the *kdp* operon is activated by the KdpDE TCS in response to osmotic stress and adjusts the potassium content of the cytoplasm to maintain turgor pressure (Wood, 1999).

The *kdpDE* genes of *S. coelicolor* appear to be the first two of a four-gene operon, with the other genes encoding a hypothetical and a putative membrane protein. This operon is convergent with another two-gene operon encoding putative potassium uptake proteins showing homology to *E. coli* TrkA. In the actinomycete *M. tuberculosis* the *kdpDE* genes are divergent from the *kdpFABC* operon and expression of the operon is highly induced by low potassium concentrations and depends on KdpDE. In *M. tuberculosis*, KdpD interacts with two lipoproteins, LprJ and LprF, which appear to act as accessory proteins positively regulating KdpD and enhancing the expression of the *kdpFABC* operon (Steyn *et al.*, 2003). The proximity of the *S. coelicolor* operon designated *kdpDE* to putative potassium uptake genes strongly suggests that they do indeed encode homologues of the *E. coli* KdpDE TCS, that these genes are regulated by KdpDE, and that they encode a potassium transporter. Interestingly, the *kdpFABC* operon in *S. coelicolor* is in a separate location on the chromosome and it will be interesting to see if these genes are regulated by KdpDE.

AbsA. The AbsA1-2 TCS was identified through the analysis of mutations that blocked production of actinorhodin, undecylprodigiosin, calcium-dependent antibiotic and the

plasmid-encoded methylenomycin, the four antibiotics produced by *S. coelicolor* (Adamidis *et al.*, 1990). The point mutations accounting for this phenotype were all located in the *absA1* (SK) gene, whereas deletion of either *absA1* or *absA2* resulted in hyperproduction of actinorhodin and undecylprodigiosin, known as the Pha (precocious hyperproduction of antibiotics) phenotype (Brian *et al.*, 1996). This implies that the original point mutants were gain-of-function mutations that led to constitutive phosphorylation of the RR. The similarity of the phenotypes for both the *absA1* and *absA2* deletion strains suggests that AbsA2 is not subject to phosphorylation by other SKs (i.e. cross-talk). Mutation of the conserved His-202 residue, the proposed site of autophosphorylation, resulted in a strain exhibiting the Pha phenotype, although the strain produced undecylprodigiosin before actinorhodin, rather than simultaneously as seen in the *absA1* deletion strain, and neither antibiotic was overproduced to the same levels as seen in the *absA1* background. No alternative site of phosphorylation could be identified on AbsA1 and the differences between the two strains have not yet been explained.

AfsQ. Closely related to the *cseBC* TCS of *S. coelicolor*, the *afsQ* genes were identified by screening *Kpn*I-digested fragments of *S. coelicolor* genomic DNA for their ability to stimulate actinorhodin production in *S. lividans* when introduced on a multicopy plasmid. The *afsQ1* (RR), but not the *afsQ2* (SK), gene stimulated actinorhodin production in *S. lividans*. Deletion of the *afsQ* genes in *S. coelicolor* had no effect either on the normal growth cycle or on antibiotic production (Ishizuka *et al.*, 1992). Since the original work was published the availability of the genome sequence has revealed that not only are the AfsQ1 and 2 proteins homologous to CseC and B (respectively) but they are also divergently transcribed from a gene encoding an ECF sigma factor that is highly similar to σ^E. There is a third gene in the *afsQ* operon, encoding a lipoprotein that may be an accessory protein to the AfsQ TCS (discussed below). Like the *vanRS* and *absA* transcripts, the transcript encoding all three *afsQ* genes in both *S. lividans* and *S. coelicolor* is leaderless (Ishizuka *et al.*, 1992; M. I. Hutchings & M. Buttner, unpublished).

CutRS. CutRS was the first TCS to be identified in the streptomycetes. It was isolated in a screen for DNA fragments that could restore melanin production in a *melC1-132* mutant strain of *S. lividans* (Tseng & Chen, 1991). Insertion mutagenesis of either *cutR* or *cutS* in *S. lividans* resulted in overproduction of actinorhodin both on solid and in liquid medium, and overexpression of *cutRS* in *S. coelicolor* repressed production of actinorhodin in the same way as the AbsA TCS (Chang *et al.*, 1996). As the authors of this work pointed out, the AbsA and Cut systems may act in some kind of hierarchical manner, otherwise disruption of both loci would be required to relieve the repression of actinorhodin production. Cross-talk between the two systems can be ruled out for the same reason, since knocking out either of the RRs, AbsA1 or CutR, resulted in actinorhodin overproduction (Brian *et al.*, 1996; Chang *et al.*, 1996).

ChiRS. The *chiRS* operon was first identified and characterized in *Streptomyces thermoviolaceus* (Tsujibo *et al.*, 1999) and subsequently in *S. coelicolor* on the basis of homology (Bentley *et al.*, 2002). In both organisms the *chiRS* genes are next to the *chi40* gene, which encodes a chitinase, an enzyme that hydrolyses chitin into chito-oligosaccharides before further enzymic breakdown results in the production of *N*-acetylglucosamine, a source of carbon and nitrogen. The model proposed suggests that ChiS is autophosphorylated at His-1199 in response to chitibiose (a dimer subunit of chitin) and subsequently transfers the phosphoryl group to Asp-54 of ChiR, which then binds to the *chi40* promoter to activate the expression of Chi40 (Tsujibo *et al.*, 1999).

Phenotypic and expression analysis

Recent microarray analysis of growth-phase-dependent gene expression and the regulation of antibiotic biosynthetic pathways in *S. coelicolor* revealed that the expression of two operons encoding group II TCSs is coordinated with the expression of the *red* gene cluster. These TCS genes were subsequently designated *ecrA1* (SCO2518) and *ecrA2* (SCO2517), *ecrE1* (SCO6421) and *ecrE2* (SCO6422) for expression coordinated with red. Expression of EcrA1-2 is also growth phase dependent (Huang *et al.*, 2001) and an EcrA1-2-deficient strain produces less undecylprodigiosin than wild-type *S. coelicolor* (Li *et al.*, 2004). In addition, the *ecrA1* SK gene was identified in a screen for developmentally impaired mutants of *S. coelicolor* using the transposon Tn*4560* (P. A. Hoskisson & M. Buttner, unpublished), strongly suggesting that this TCS is involved in development. One other TCS (SCO3654/SCO3653) was found to be developmentally impaired in this screen (P. A. Hoskisson, J. Towle & M. Buttner, unpublished), although in this case expression was not coordinated with *red* or growth phase dependent (Huang *et al.*, 2001). Finally, expression of four more genes encoding TCSs [SCO0203/SCO0204 and SCO5748/5749 (*osaAB*)] was found to be growth phase dependent. Disruption of the *osaB* (RR) gene results in a strain that is sensitive to osmotic change, is conditionally bald (i.e. cannot raise aerial hyphae), and has uncoordinated antibiotic production, with actinorhodin and undecylprodigiosin massively over-expressed (Bishop *et al.*, 2004). The *osaA* (SK) and *osaB* genes are separated by 500 bp, and are not co-transcribed (Bishop *et al.*, 2004). However, the SK primary sequence shows strong similarity to putative osmosensing SKs from yeast (Tao *et al.*, 2002).

Unpaired histidine kinases

The 17 unpaired SKs span all four groups and include the single group I (SCO1217) and the two group IIIb (SCO1596 and SCO7297) SKs. None of these unpaired SKs have been characterized, although the group IIIb SK SCO1596 was identified in a screen for developmentally impaired mutants

(P. A. Hoskisson & M. Buttner, unpublished), suggesting that it may be involved in development. Another interesting unpaired SK is encoded by one of the *S. coelicolor* conservons (SCO6794 or *cvnA7*). The 13 conservons of *S. coelicolor* each consist of a cluster of four genes (*cvnA–D*), of which *cvnD* encodes a nucleotide-binding protein, *cvnB* and *cvnC* encode proteins of unknown function, and *cvnA* encodes a membrane protein with weak similarity to SKs (Bentley *et al.*, 2002), although, judging by our analysis, only *cvnA7* encodes a true SK. The presence of so many unpaired kinases in the genome suggests that they might specifically activate other RRs, responding to signals that differ from that of the RR's cognate kinase. This would allow subsets of genes to be activated in response to a wider range of environmental stimuli than might be sensed by a single SK.

Orphan response regulators

Typically, the N-termini of RRs have several conserved residues which form the phosphorylation pocket; these conserved residues include two adjacent aspartates near the N-terminus of the protein (DD), an aspartate in the middle of the N-terminal domain, usually close to position 54 (D54), a hydroxylated residue at position 82, normally serine or threonine (S/T82) and a lysine residue near the end of the N-terminal domain, close to position 105 (K105). Here the RRs are characterized as typical where they contain the conserved residues of the phosphorylation pocket (including at least one of the adjacent aspartates) or where they have been shown to be dependent on phosphorylation experimentally and the atypical RRs are those which lack two (both DD and another residue) or more of the conserved residues which make up the phosphorylation pocket. Of the 13 orphan RR genes in the *S. coelicolor* genome, seven have typical phosphorylation pockets, five have atypical phosphorylation pockets and a single RR (BldM) has a 'pseudo'-phosphorylation pocket (Table 2). BldM contains all the conserved amino acids but has been demonstrated to be active in the absence of phosphorylation (Molle & Buttner, 2000). Only five of these orphan RRs have been studied experimentally and the findings are described below.

Atypical orphan response regulators

BldM. The BldM N-terminal phosphorylation domain is apparently typical; indeed the original point mutations isolated in the *bldM* locus lay in the putative phosphorylation pocket (Molle & Buttner, 2000). However, the conserved Asp-54 residue is not required for function. Replacement of this residue with unphosphorylatable residues such as asparagine (Asp-54N) or alanine (Asp-54A) did not affect its function, with wild-type levels of aerial hyphae and spores formed (Molle & Buttner, 2000). An Asp-54N substitution in CheY resulted in an active

Table 2. Orphan response regulators

Conserved residues refers to those in the N-terminal phosphorylation pocket; the presence or absence of these residues was used to classify these pockets as either typical or atypical (see text). Phenotypes and functions are given where available, along with references describing the experimental evidence.

Name	Conserved residues				Function/phenotype	Phosphorylation pocket	Reference
	DD	D54	S/T82	K105			
SCO1654	Y	Y	Y	Y	Unknown	Typical	None
SCO2281	½	Y	Y	Y	Unknown	Typical	None
SCO3008	Y	Y	Y	Y	Unknown	Typical	None
SCO3134	Y	N	N	Y	Unknown	Atypical	None
SCO3144	Y	N	N	N	Unknown	Atypical	None
SCO3818	Y	Y	Y	Y	Unknown	Typical	None
SCO4159, *glnR*	½	Y	N	Y	Required for expression of the GSI-like enzyme, GlnA	Typical	Fink *et al.* (2002)
SCO4768, *bldM*	Y	Y	Y	Y	Required for formation of aerial hyphae	Pseudo	Molle & Buttner (2000)
SCO5506	N	N	N	N	Unknown	Atypical	None
SCO5881, *redZ*	N	N	?	?	Pathway specific activator for the antibiotic, Red	Atypical	Guthrie *et al.* (1998)
SCO6029, *whiI*	N	Y	Y	N	Required for normal septum formation in aerial hyphae	Atypical	Ainsa *et al.* (1999)
SCO6364	½	Y	Y	Y	Unknown	Typical	None
SCO6685, *ramR*	½	Y	N	Y	Developmental	Typical	Keijser *et al.* (2002); O'Connor *et al.* (2002); Nguyen *et al.* (2002)

protein *in vivo* as a result of phosphorylation of the adjacent Ser-56 residue (Bourret *et al.*, 1990; Appleby & Bourret, 1999). Phosphorylation at alternative residues has also been reported in FixJ of *Rhizobium meliloti* and NtrC of *E. coli* (Reyrat *et al.*, 1994; Moore *et al.*, 1993). However, no potentially phosphorylatable hydroxyamino acid residues are found adjacent to Asp-54 in BldM, suggesting that BldM has a pseudo-phosphorylation pocket.

WhiI. The *whiI* mutant is unable to sporulate and the lack of grey spore pigment gives a white colony phenotype. The *whiI* gene encodes an orphan RR that is required for normal septum formation early in the development of aerial hyphae. The phosphorylation pocket, however, is atypical, lacking the lysine residue and one aspartate conserved in conventional phosphorylation pockets (Ainsa *et al.*, 1999). This prompted the suggestion that *whiI* is regulated via another means, such as small ligand binding, or interaction with another protein. Ainsa and co-workers speculated that the presence of the conserved Asp-69 in the putative phosphorylation pocket might permit phosphorylation as part of a phospho-relay signal transduction system similar to that of the Spo0A sporulation cascade of *B. subtilis* (Hoch, 1993).

RedZ. RedZ, the pathway-specific activator for undecylprodigiosin (also known as Red), lacks the paired aspartate residues and the lysine residue conserved in the phosphorylation pocket of conventional response regulators (Guthrie *et al.*, 1998). Despite this, there is experimental evidence to suggest that RedZ is regulated post-translationally (J. White & M. J. Bibb, personal communication).

Not just RedZ. The atypical nature of several *S. coelicolor* RRs, either lacking the conventional phosphorylation pocket, or having a pseudo-phosphorylation pocket, raises the possibility that these are regulated by other means. DnrN, from *Streptomyces. peucetius*, is a response regulator required for daunorubicin biosynthesis. It has a typical phosphorylation domain, but DnrN with an Asp-55N mutation is active *in vivo*, and the mutant protein binds its target site with equal affinity to the wild-type protein (Otten *et al.*, 1995; Furuya & Hutchinson, 1996). The possibility of post-translational regulation mediated by a co-regulator interacting with the phosphorylation pocket of the atypical response regulator has been suggested for both BldM and RedZ (Molle & Buttner, 2000; J. White & M. J. Bibb, personal communication). Interestingly, all the above examples of atypical RRs lack a paired, cognate sensor kinase. This suggests that lack of the cognate kinase might result in regulation of these proteins by a means other than Asp-54 type phosphorylation.

Typical response regulators

RamR. The *ram* (rapid aerial mycelium) cluster plays an important role in the development of *S. coelicolor*. The *ram* genes encode a membrane-bound kinase (RamC), a small protein (RamS), the subunits of an ATP binding cassette (ABC) transporter (RamAB), and a RR (RamR). The *ramC* and *ramR* genes are required for production of aerial hyphae but are dispensable for vegetative growth (O'Connor *et al.*, 2002), and over-expression of RamR overcomes the bald phenotype of all tested *bld* mutants of *S. coelicolor* (Nguyen *et al.*, 2002). A homologous gene cluster *amf* is found in *Streptomyces griseus* and *Streptomyces avermitilis*. In *S. coelicolor*, the *ramR* gene product has a typical phosphorylation pocket and is essential for transcription of the *ramCSAB* cluster (Keijser *et al.*, 2002). In *S. griseus*, the integrity of the phosphorylation pocket and the conserved aspartate residue have been shown to be essential for the function of the RamR homologue, AmfR, suggesting that phosphorylation of the aspartate is required for activity (Ueda *et al.*, 1993). However, this has not been demonstrated experimentally in *S. coelicolor*. A caveat to this is that the sequence similarity between the *S. coelicolor ram* cluster and the *amf* cluster of *S. griseus* is lower than would be expected for orthologous genes (Keijser *et al.*, 2002).

GlnR. The *S. coelicolor* genome contains five glutamine synthetase (GS) homologues: a GSI of the β-subtype, three of the GSI-α-subtype, and a GSII-type enzyme, a type normally associated with eukaryotes (Fink *et al.*, 2002; for a full discussion of GS types see Brown *et al.*, 1994). The OmpR-like RR, GlnR, is required for transcription of the gene encoding the GSI-like enzyme, *glnA*, in *S. coelicolor* (Fink *et al.*, 2002; Wray & Fisher, 1991), and a *glnR* null mutant exhibits glutamine auxotrophy. A close homologue of GlnR, named GlnRII, is required for GSII transcription. GlnRII shows significant similarity to GlnR in the C-terminal DNA binding domain but lacks the N-terminal CheY domain for SK interaction. In addition, the conserved phosphorylatable aspartate, and the conserved serine/threonine, and tyrosine residues in the phosphorylation pocket, also postulated to be involved in phosphotransfer, are all absent in GlnRII (Fink *et al.*, 2002). Thus it would appear that GlnRII is not a functional homologue of GlnR and is not a true member of the RR family.

The remaining five orphan RRs with typical phosphorylation pockets have yet to be characterized (Table 2). However, BldM is an example of a RR that has a typical phosphorylation pocket and does not require D54 for activity. Therefore, while Table 2 lists these RR domains as typical or atypical, only experimental analysis will determine whether or not these proteins are true members of the RR family.

Accessory proteins

It has recently become apparent that in addition to protein–protein interactions with each other the RR or SK proteins of many two-component systems also interact with accessory proteins that either positively or negatively regulate their activity. In some cases this has been shown

simply through the use of two-hybrid systems to screen libraries for interaction with either the SK or RR (e.g. Steyn *et al.*, 2003), while in an increasing number of cases both negative and positive regulators of two-component systems have been reported. Thus, what once appeared to be relatively simple signalling pathways are likely, in some cases, to be more complex and to involve more than just two components. Regulation of these systems can occur at several levels: modulation of the SK sensor domain, for example CpxP of *E. coli* (DiGiuseppe & Silhavy, 2003); inhibition of SK autophosphorylation, for example KipI of *B. subtilis* (Wang *et al.*, 1997); phosphatase activity against the SK, for example SixA of *E. coli* (Ogino *et al.*, 1998); or phosphatase activity against the RR, for example CheZ of *E. coli* (Parkinson, 2003). In the sporulation signal transduction cascade of *B. subtilis* the response regulator Spo0F is subject to regulation by the RapA, B and C phosphatases while Spo0A is regulated by the Spo0E, YnzD and YisI phosphatases (Perego, 2001).

In *S. coelicolor*, the genes encoding six of the group IIIa SKs, including CseC and AfsQ2, lie in operons with genes encoding predicted membrane proteins, secreted proteins or lipoproteins. The *cseA* gene encodes a membrane protein, whereas *afsQ3*, SCO3011, SCO5305 and SCO7535 encode putative lipoproteins, and SCO7232 encodes a putative secreted protein. Five of the six genes also lie in operons with a RR gene, while a single operon encodes only a SK (SCO5304) and a putative lipoprotein (SCO5305). Analysis of the primary sequences of the six gene products using TopPred II predicts that each protein has a single N-terminal transmembrane helix. Furthermore, the C-terminus of each protein is predicted to be extracytoplasmic (Claros & von Heijne, 1994; http://bioweb.pasteur.fr/seqanal/interfaces/toppred.html). Although these proteins have little homology to one another at the primary sequence level, this analysis suggests that they have similar structures and may therefore have similar functions.

The best-studied of the six SKs in this subgroup is CseC, which is encoded by the last gene in the *sigE* operon. The *sigE* gene encodes a well-studied extra-cytoplasmic sigma factor, σ^E (Hong *et al.*, 2002), and the *sigE* operon promoter is positively regulated by the CseB/C two-component system (Paget *et al.*, 1999). Recent work has shown that CseA is a negative regulator of *sigE* transcription, that it is localized to the plasma membrane by the first N-terminal 21 amino acids, and that the C-terminus is extracytoplasmic (M. I. Hutchings, H.-J. Hong, E. Leibowitz & M. J. Buttner, unpublished). Thus the TopPred II predictions, for this protein at least, are correct and imply that CseA must interact with the sensor domain of CseC, or another component of the signal transduction system acting upstream of CseC, in order to negatively regulate transcription of the *sigE* operon. It is interesting that the CseA protein has no homologues in the databases, although it seems possible that the lipoproteins encoded by the other operons discussed here are structural homologues, i.e. they also function as accessory proteins to their linked TCS. Studies are under way to analyse the AfsQ proteins and to determine if CseA interacts with CseC.

Future prospects

The recent development of a rapid knockout strategy in *S. coelicolor* (Gust *et al.*, 2003) will make it possible to disrupt each of the TCSs in turn in an effort to determine which are essential for survival and to gain some clues as to their function through characterization of the mutant phenotypes, as was recently reported for *E. coli* (Zhou *et al.*, 2003). Coupled with the availability of *S. coelicolor* whole-genome microarrays, rapid progress should now be possible in further characterizing the 67 TCSs, 13 orphan RRs and 17 unpaired SKs. Since the streptomycetes produce around 80 % of the commercially available antibiotics, understanding the TCSs of this model organism may also provide insights into increasing antibiotic production in closely related, industrially important actinomycetes.

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