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NOTE

Identification of σ^B-Dependent Promoters Using Consensus-Directed Search of *Streptomyces coelicolor* Genome

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σ^B plays an important role in both osmoprotection and proper differentiation in *Streptomyces coelicolor* A3(2). We searched for candidate members of the σ^B regulon from the genome database, using the consensus promoter sequence (GNNTN$_{14\text{-}16}$GGGTAC/T). The list consists of 115 genes, and includes all the known σ^B target genes and many other genes whose functions are related to stress protection and differentiation.

Key words: osmotic stress, differentiation, sigma B (σ^B), *Streptomyces coelicolor*

In gram-positive bacteria, an alternative sigma factor, sigma B (σ^B), regulates expression of numerous genes under environmental stress conditions and upon entry into the stationary phase. σ^B regulates expression of a large number of general stress operons in *Bacillus subtilis*, contributing to the transcription of more than 200 genes involved in heat, acid, ethanol, salt, and oxidative stress resistance (Hecker and Völker, 1998; Price, 2000; Petersohn *et al.*, 2001). Its ortholog σ^B is also involved in stress resistance in *Listeria monocytogenes* and *Staphylococcus aureus* (Becker *et al.*, 1998; Kullik *et al.*, 1998; Wiedmann *et al.*, 1998), virulence in *Bacillus anthracis* (Fouet *et al.*, 2000), and the formation of adherent biofilm in *Staphylococcus epidermidis* and *S. aureus* (Rachid *et al.*, 2000; Knobloch *et al.*, 2001). Among high-GC Gram-positive bacteria, a σ^B-like factor is known to be important for virulence in *Mycobacterium tuberculosis* (Chen *et al.*, 2000).

In *Streptomyces coelicolor*, a model organism of high GC gram-positive bacteria that undergoes fungi-like differentiation, nine σ^B-like sigma factors (σ^B, σ^F, σ^G, σ^H, σ^I, σ^K, σ^L, σ^M, σ^N) are predicted from the genome sequence (Kelemen *et al.*, 2001; Bentley et al., 2002; Hahn *et al.*, 2003). Among them, σ^B was demonstrated to play an important role in osmoprotection and formation of the aerial mycelium (Cho *et al.*, 2001), partly by regulating the transcription of the *catB* gene, which is also responsible for differentiation and osmoprotection (Cho *et al.*, 2000). The *sigB* gene is induced dramatically from the *sigB*p1 promoter immediately after osmotic shock *in vivo*, in a σ^B-dependent manner. RNA polymerase holoenzyme with σ^B directs transcription from *sigB*p1 and *catB* promoters *in vitro*. Therefore, it was suggested that σ^B is a major sigma factor that controls the global transcription of osmotic stress and differentiation in *S. coelicolor*. However, not many σ^B-dependent genes have thus far been identified. In order to understand the range of cellular functions that are under σ^B control in *S. coelicolor*, the function of its target genes needs to be verified.

In our initial effort to find σ^B-dependent genes, we used a consensus promoter-based search for new σ^B targets from the current database (http://www.sanger.ac.uk/Projects/S_coelicolor/), selected a list of candidate genes, and verified some of them by S1 nuclease mapping as new members of the σ^B regulon of *S. coelicolor*.

The promoter consensus search was performed using a pattern search algorithm of OMIGA™ 2.0 program (formerly provided by Oxford Molecular Group, UK, but currently by Accelrys, http://www.accelrys.com). The consensus promoter sequence (GNNTN$_{15\text{-}16}$GGGTAC/T), extracted from *catB* and *sigB*p1 promoters, very closely resembles that of σ^B-specific promoters of *B. subtilis*, (Cho *et al.*, 2000, 2001). Using this sequence pattern, we searched the whole genome, and selected those putative promoters located within 500 bp of annotated open reading frames. Seventy five promoters (including *catB*p and *sigB*p1) were initially collected. Considering that RNA polymerase holoenzymes are tolerant of moderate varia-

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Table 1. σ^B-dependent promoters searched by hte consensus pattern (GNNTN$_{14\text{-}16}$GGGTAC/T) from *S. coelicolor* A3(2) M145. All 118 promoters from 115 genes containing putative σ^B-dependent promoter(s) were listed by their potential function. The tentative -35 and -10 regions were underlined. *SCO1468* has four σ^B consensus promoters, which were named p1, p2, p3, and p4. The genes whose dependence on σ^B was verified by individual RNA analyses were indicated by S1.

ORF	*Gene*	Function or nearest homolog	Regulatory or potential sequence	Other Evidence	Reference
Stress response					
SCO0666	*catB*	catalase B	ATGCCTCGACTCCCGAAGGCTGGGTACGCGGTCACGGCCCACCACG	S1	Cho *et al.*, 2000
SCO0885	trxC	thioredoxin C	TGGTGTGCGGCGTGGCCACATGGGTACGTGCGCGGAATACCCACCAG	S1	This study
SCO3669	SCH44.09c	DnaJ, heat shock protein	CTGCGTGGCGATCCTGCAGCCGGGGTACCGGATCGGCGAACGCACCA		
SCO6531	SC5C7.16	OsmC, ATP/GTP-binding protein	ACGGCTGGTGCGACGTCCTGCGGGTACGGCGCGAGCACGCCTACCTG		
SCO7156	SC9A4.18C	hypothetical protein similar to UspA	CGGCCTTCGCCCAGAGCGTGGGGTACCTGCTGGCGATCCCGGGACCG		
SCO7339	SC4G10.18c	SgaA	CCGTTTCGGCGCCATGTGCGGGGTACTCGCCAGGCGCCCGCCGCACT	S1	This study
Replication/Transcription					
SCO0600	sigB	*RNA polymerase sigma factor*	GGGAGTGAGCCGGATCAGTGAGGGGTACGACTGGCCGGCGGTCCTG	S1	Cho *et al.*, 2001
SCO0869	SCM1.02c	anti-anti-sigma factor	GTGCTTGTGCTTGGGGCGGCGGGTACGCCCGCGTCGGCCGATTGTCG		
SCO1534	SCL2.24	DNA polymerase III	CGGCGTACGCCGCAAACAGCCCGGGTATCACCGAACCGGGACAAGGG		
SCO1813	SCI28.07	GntR-family transcriptional regulator	GCGCGTGGCGGACGCCTCGCCGGGTACCTCGGACACAACGCCCCCGG		
SCO3067	SCE25.08c	ArsI, anti-anti-sigma factor of sigI	GGGAGTGTCGACGTCGCTTGGTGGGTACGCGATCCGTCGAGCCGGGG	S1	Lee, 2003
SCO4495	SCD35.02	DNA polymerase-related protein	ACGGGTCCGTACCCCTACTGCGGGTACCCGCGTTCCATGGCCACCGA		
SCO5244	prsH	Anti-sigma factor of sigH	ACGGTTCGACCGTCTGACGTCTGGGTACGTCAACCCGGCGCGCGC	S1	Lee, 2003
SCO5405	SC8F4.09C	*probable transcriptional regulator*	GAGCGTCGGGAGGCCGGTCGGGGGTACCGGGGCTTCCCGGACAGCTC		
SCO5933	SC7H1.03C	membrane protein similar to RsbU	CCGGGTGGACTCACTTCGGCACGGGTACGGCCAGCCTCCCTCCGGCA		
SCO5934	SC7H1.04	CnrH, ECFsigma factor	CCGGCTGCCGGACATGGACAGGGTATGGAACTGAACGACGCCGGTCC		
SCO6669	SC5A7.19c	transcriptional regulator	GCGCGTTCTCGCCCCAGGGCGGGTATCAGACGCTCTCCTACGTCCTG		
SCO7220	SC2H12.19c	RsbU-like protein with ATPase damain	GGGGCTCTTCCGCACGGGTTCGGGTACGGCCCCGGCACTGTGCCGG		
SCO7277	SC5H1.15c	putative regulator of sigL	TGGACTGCGACGCGCGACTCGGGGTACGAGGACAGGTGCAGCAGCGT	S1	This study
SCO7278	SC5H1.14	SigL, sigB-type sigma factor	AGGCATGAGCCGCCGCGACCCGGGTACCCGCAAGCGCGAAAAACCTA	S1	Lee, 2003
SCO7325	SC4G10.04c	*RsbV, anti-anti-sigma factor*	AGGTGTGGAGTCGGGATCCGGGGGTATCCGGGCGGCGCGGCGGACCA	S1	Lee, 2003
Signal transduction					
SCO1468	SCL6.25c	serine/threonine protein kinase p1	ACGGGTACGGCCCCCGGGTCGGGTACGGCGGAGGGGATGCGTCATGA		
		serine/threonine protein kinase p2	ACGGCTGCGGCGCCCGGGACGGGTACGGCCCCCGGGACGGGTACGGC		
		serine/threonine protein kinase p3	ACGGTTACGGCGCCCGGGACGGGTATGGCGCCCGGGGCGGCTGCGGCG		
		serine/threonine protein kinase p4	ACGGTTACGGCGCCCGGGACGGGTATGGCGCCCAGGACGGGCATGGC		
SCO3390	SCE126.08c	two-component sensor kinase	TGGTGTCACAAGCCCAGGCTAGGGGTACAGGAGAGCGGTCCCTCCAC		
SCO3638	SCH10.16c	*two-component system regulator*	GCGGGTCCGACGGACGACGGCGGGTACGAGGTGGCGGTCTTCCTGCC		
SCO5794	SC4H2.15	kinase phosphohydrolase, RelA/SpoT	TCGACTCCGCCTGGCTGGAAGGGGTACTCGCCTGAACCACGACCCGG		
Translation					
SCO0436	SC51A.14	50S ribosomal protein L32	CCGCCTACGGCTCCCCGGGACGGGTACGGCTCAACGTCGGGTGCCCC		
Differentiation/Cell cycle					
SCO3404	SCE9.11c	FtsH2, cell division protein	GCGGCTGAGGGCCGCCACGCTGGGGTACCGTCAGAAGAACTGTCTTA		
SCO3885	SCH24.07	GidB-family, glucose-inhibited cell cycle protein	CCGCCTGATGCTCGACATCGCGGGTACCGCGCCGACAAGCGCGCCG		
SCO7289	SC5H1.03	SsgC, ssgA-like sporulation protein	GAGCATCTTGCCGGTGGGCGGGGTACTAGAGGGCTCTACGTCCGTCC	S1	This study
Seondary metabolite biosynthesis					
SCO0392	SCF62.18	methyltransferase, similar to daunorubicin biosynthesis gene	CGGCCTGGAGCGCCTGCTGGAGGGGTACCGCACCGCGATCGCGGGGC		
SCO5881	redZ	pathway-specific regulator of prodigiosin biosynthesis	ACGTGTCCTTCTGAGCGGAGCGGGTACGAGGGACGGGTACGGGAGC		
Influx/Efflux					
SCO1045	SCG20A.25	metal-associated protein	TTGCGTTGGGTACCCCCTAGGGGTATACATGGATGGGCCGGGGGCGG		
SCO2071	SC4A10.04c	NA+/H+ antiporter	TGGGATTTTACGAACGTCTGGGGTACGAGGCATACGGACCCGAGTT		
SCO2829	SCE20.03	amino acid ABC transporter	TCGCCTACGCGGTGGAGCACGGGGTACAAGGAGGACGACATCCTGAT		
SCO6062	SC9B1.09	ABC trasnporter ATP-binding subunit	CAGCTTCTGCCAGACCCGAAGGGTATTCGGCGTTTTGATACGGATGG		
SCO6720	SC5F2A.03c	ABC transporter	CCGCGTCCCACGTATCCCACGTGGGTACGTCGCGTGCGACCTCGGCG		
SCO7166	SC9A4.28C	putative sugar transporter	AGGACTCCTCGTTCCGCACCGGGTACCCCTACAAGCTCTCCCCGCTG		
Carbohydrate metabolism					
SCO1388	SC1A8A.08c	mannose-1-phosphate guanyltransferase	TCGGATGGGCGTTCGCCGGATGGGGTACAACCCGTTATTGG		
SCO1936	SCC22.18	Tal2, transaldolase	GTGGTTCGAGCAGCAGGACCAGGGGTACCGGGACAGCGTCCTGCCCC		
SCO1947	SCC54.07C	Gap1, glyceraldehyde-3-phosphate dehydrogenase	GCGGGTACGGACCGCCCTGCCGGGTATGGGGCGTCTCGATGTCACCC		
SCO2371	SCC8A.29	AceE2, pyruvate dehydrogenase E1 component	GCGTATTTGGGAACCATGTGTGGGTACGAGACTCGAATGAACGTGCC		
SCO2531	SCC117.04	beta-glucosidase	GGGCGTTGACTTCGCTCACCACGGGTACGGATGATCCGCGCATGGCG		
SCO4787	SCD63.19	aldolase	GAGGGTGGCGAGCACGTGGTGCGGGTACGCGCCGAAGCGTGCCTCGA		
SCO5208	SC7E4.05c	putative inositol monophosphatase	GCGACTTGCTGCCAGATTTGACGGGTATGGGAGCACTGTCAAGGGTC		
Turnover					
SCO1648	SCI41.31c	Arc, AAA ATPase	GGGGTTCCCCCCACGTTCCGTGGGTATTCCCCTGGCGTGAGGGAGAG		
SCO1647	SCI41.30c	putative proteasome component	GCGGCTGCGGGTGCCCACACCGGGTACCCGCAGCCGACTGTTTTCCG		
Cell wall/outermembrane biogenesis					
SCO3194	SCE22.11	putative lipoprotein	CGGTGTTCGCCCTGGCGACCGGGTACGTCCGCTCGACGCCGGGCAG		
SCO3246	SCE29.15c	FabH3, acyl carrier protein synthase III	CCGGGTACCGGACCGAGCCCGGGTACCGGACCGGCCCCGGGCACCGT		
SCO5560	SC7A1.04	DdlA, D-alanine-D-alanine ligase	CTGTCTCGAGGCACCGCGGGCGGGTACTCTCAACGCGATATGAGCAC		
SCO5753	SC7C7.08	PgsA, phosphatidylglycerophosphate synthase	GCGCGTCGGGGCGACCGTGCGGGTACTCGTCGAGTCCGTGGACCCGG		
SCO6468	SC9C7.04C	Psd, putative phosphatidylserine decarboxylase	ACGCCTTCCGGATCCACGGCGGGTACGGCTTCTCCAAGGAGTACGAG		
SCO6717	SC4C6.27c	putative fatty acid desaturase	GCGGGTCTCGGGGCCCGTGTCGGGTACCGCCGGACGGTGGCTGATCA		
SCO6487	SC9C7.23	putative aminoacylase	TGGCCTGCGAGGACGCGCTCGGGTACGCGCCCGAGGCGCGCAAGGTC		

Table 1. Continued.

ORF	Gene	Function or nearest homolog	Regulatory or potential sequence	Other Evidence	Reference
Oxidation reduction/electron transport					
SCO0111	SCJ11.40	oxidoreductase	GTGCGTCGACGGACTGTCGCGGGTATGCCGACGTCCTCGGGTGAGAC		
SCO1773	SCI51.13c	L-Alanine dehydrogenase	CGGTGTGCCCGCGCTTGAGCGGGTATGCCTTGTAATAGCCCATCCTC		
SCO2397	SC4A7.25c	putative oxidoreductase	TGGTGTGGCCATGGAATCCAGGGTACCCATTTGTTTGACAAGCTGA		
SCO3092	SCE41.01c	oxidoreductase	CAGGATCCTCGTAGTAGGCGGTGGGTACGTAGGCCTGTACGCAGCTC		
SCO3271	SCE39.21	putative dehydrogenase	GAGGCTGGCTACCGGGAGAAGTGGGTACAGCACGACTTCCCCGGCGC		
SCO5857	SC9B10.24C	FAD-dependent oxidoreductase	ACGCCTCCGGGGCGCGTGCCGGGTACGGGGTTCCGGCGGTGGCCGGG		
SCO6496	SC1E6.05	probable dehydrogenase	GGGGGTGGGTCCGGGGCACCGGGTACCCGGGCGCGGTGCCGGA		
SCO7374	SC10G8.01c	truncated oxidoreductase	TCGCTTCGATCGGTGGCGCCCGGGTACCCGGCGGCCGGC		
Coenzyme biosynthesis					
SCO0185	crtB	geranylgeranyl pyrophosphate synthase	ACGCGTGTCCGTACACCACCGGGTACTGGGCGTTCCTCGACCGGCAC		
SCO0826	SCF43A.16	methyl transferase similar to UbiE	GGGAGTGCATGACGCGGCACGGGTATGTCTCCCGCTACTACGAGGCC		
SCO5250	gtr	polyprenyl synthetase	CCGTGTTCCCGCCCTCCCGGGCGGGTACACCCCCGGGGGTACGGA		
Transposable element					
SCO0099	SCJ11.28	transposase	GGGCGTCGGACAGGTCGCTGGGGTACGGCTTGCGCTCACTCACGGCA		
SCO6911	SC1B2.17	IS element	GGGCGTCGGACAGGTCGCTGGGGTACGGCTTGCGCTCAC		
Secreted protein					
SCO1843	SCI8.28c	secreted protein	GTGCGTGCCCGGCGGCTGCGCGGGTACCGGCTACGGGACACGAAGAC		
SCO2461	SCC24.32	secreted protein	TCGCATCGAACGCGTCGGCCTGGGTATTCGCCAGGGGGCGCGCGCTC		
SCO2766	SCC57A.37	secreted RNase	TCGGCTGTACGCGACGATGCGGGTACTGAAGTCCCTCACCGAGCCTT		
SCO5530	SC1C2.11	secreted protein	ATGTCTGATTGAGGTCCATTCGGGTACGTTCGAACGTATGAA		
SCO7237	SC7A12.04c	secreted protein	GCGGGTTCGTCACGCGGGCCGCGGGTACCCGCGCTCCATGACCAACG		
Membrane-associated protein					
SCO1251	2SCG1.26	membrane protein	CAGCATGCCTGGCGGGCCGCCGGGTACGAGCCGCAGGAGCAGTGGC		
SCO1833	SCI8.18c	membrane protein	GTGCGTCACCAAGTCGTCCAGCGGGTACGTGCGTTGGTGGGCATGGC		
SCO2350	SCC8A.08	integral membrane protein	AGGAGTCGGTGAGGGCGGGACGGGGTATGAATTGAGTCACATCGGCG		
SCO2372	SCC8A.30C	small hydrophobic protein	AGGCATGACACGCCGTACGCGGGTACCCGAACTTCACGGAGAGTTC	S1	This study
SCO2580	SCC123.18C	membrane protein	CCGGGTGGTTCCTGTTGCGGGGGTACAAGCAGAAGGACGACTGAGGA		
SCO3159	SCE87.10	membrane protein	GGGGGTCGTAGCGCAGCACGACGGGTATCGCTCCGCCGTCGTCCTGG		
SCO3192	SCE22.09	integral membrane protein	GGGGGTGGTACTCATGCGTCCAGGGTACGGCTCACCCCGTGGACGAC		
SCO3802	SCGD3.03	membrane protein	CGGCATGAGGGTTGAAGCCGGGGGTACCCGCACGGAACCGGATCGGC		
SCO3872	SCH18.09c	integral membrane protein	CCGGATCGGCCGGTACCCCCTCGGGTACGGAAACGGGCGGCGGCGGC		
SCO4104	SCD17.08c	integral membrane protein	CCGGCTGACGAACCCGGCGGCGGGGTACCCGGCGCCCATGAGCACC		
SCO4200	2SCD46.14	membrane protein	TGGGGTGTCCGGCTCGTTCTGCGGGTACGGTGACCGCGTAATGACGAT		
SCO4519	SCD35.26	integral membrane protein	CTGGATCGAACCCCAGATTCGGGTATTTTTCGGAACTCATCGGACTT		
SCO4733	SC6G4.11	integral membrane protein	ACGGCTCGGTGGCCGTCGCGCTGGGTATCTCCGCGGTCGTGGGGAGC		
SCO5011	SCK15.13	integral membrane protein	TCGTATGCCGTCCCCGTCCTGGGTATCGGCTCGTTGCTCCTGATGAA		
SCO6494	SC1E6.03	membrane protein	CGGTTTCCGGCACCGCGCCCGGGTACCCGGTGCCCCGGACCCA		
SCO6527	SC5C7.12	membrane protein	CAGGCTGTCGATGCCGACGGCCGGGTACGACGGCCGCTGACCGCCGC		
SCO7431	SC6D11.27c	integral membrane protein	CCGCTTGGGCAACCTTCCTGCGGGTACTCGCAGTTCACTGGAGAGCG		
SCO7823	SC8E7.20C	membrane protein	CGGCATGACCGCTCTTGCCGGGGTACCCGCTCCCGGTTCCGTCGCCC		
Unknown					
SCO0359	SCF41.18	unknown	CGGCCTGCCGGTGAGCCCCGTCGGGTACAACGCCAACCCGGTCGCGC		
SCO0617	SCF56.01c	unknown	CGGTGTACGACGCCGAAGTTGGGGTACCCGGTGCGTCGGCACGGTCG		
SCO0759	SCF81.18	unknown	GGGGGTGCCACGGCGCCGGCCGGGTACTCGCGGCGCATGGAGTACGA		
SCO0792	SCF43.03	unknown	TTGCGTGCCCTTCCCGGGCTGCGGGTACCCGGTGCTCTCCGCACGGG		
SCO0964	SCM11.19c	unknown	CCGAGTTCATCGATTCCATCGGGTACGGCGTGGTGGACGCGGGGCTC		
SCO1029	SCG20A.09	unknown	GTGCCTCAAAGGGGCCCCTGGGGTACACGGCGGTCGACGCGACGGGG		
SCO1089	2SCG4.05c	unknown	CCGTTTCCCCCGTGCCGCGCCGGGTATACGAATCCGGCGCCCGACA		
SCO1754	2SCI34.07c	unknown	GTGGGTGCTCGGGAATGTTCGGGTACGGAATGCGTGGCCCGGGCAAG		
SCO1791	SCI51.31c	threonine, valine, serine, arginine-rich protein	TCGTTTTTCCAGGGCGCACCGGGTACCCGGCTGCGCGAGAATGACTT		
SCO1910	SCI7.28C	alanine-rich protein	AGGGGTAGCGCCGTTCGGCGCGGGGTACCCGGGGTGCTCCGAACCGA		
SCO2790	SCC105.21C	unknown	AGGCGTGCCGCCGTACCCTTGGGGGTATGGGGTTGGGAAGACCATTC		
SCO3312	SCE68.10	unknown	GTGAGTGGTCCGCCACCTCCGGGTACGCCACCCCCGGCAGGCTCGCG		
SCO3343	SCE7.10c	unknown	GTGTTTCATGGTTTGTTCTTGGGGTATCAACGGTGCCCCGCGAAAGT		
SCO3465	SCE46.22	unknown	CCGGATGTCTGGAGCACGGACCGGGTACTCGCGGCGACCCGCGCCCT		
SCO3903	SCH24.25c	unknown, 343aa	CGGCCTGATCCAGTTCAAGGTGGGTACGGGCGGTCAGGCGGCCGAGT		
SCO4254	SCD8A.27	unknown	CGGCATGCTCTGCTCTCCTCCGGGGTACGAGACAGGACGCCCCTCACG		
SCO4623	SCD39.23	unknown	CCGTCTTGGCGCCGCGGGCACGGGTACGACTGGACACAGGAACTCTC		
SCO5207	SC7E4.04C	unknown	CCGGCTCCACGACGAACTGCTCCGGGTATCTCAACCAGCGCTACTGAG		
SCO5788	SC4H2.09	unknown	GCGGCTGCGACTGACCGCGGGGGTACCGCCGGGGGGCGGCGACACGG		
SCO5813	SC5B8.03	proline-rich protein	TCGGTTTCGCGGACAACGCCGGGTATCCCTCGCCCGTGCACAAGGCC		
SCO6516	SC5C7.01c	unknown	CCGTGTCGGTCCGCGGTGTCCGGGTACGCGGTCGGCACCCCTTAAGG		
SCO6672	SC5A7.22	unknown	CGGGGTGTTGGGGCGATCATAGGGGTACGCGCGGCCCCCAGCGCAAC		
SCO7041	SC4G1.07	alanine, leucine, aspartate-rich protein	CAGGGTTGCGCCGTGGGTGACCGGGTACGCGAGGCTGGACCGCGTCC		
SCO7136	SC4B10.37	conserved hypothetical protein	ATGGGTCTTCCTCTCGGCCGGGGTACGGGGACCGGGCGGAGCCCGAC		
SCO7238	SC7A12.05	unknown	ATGCGTTGGTCATGGAGCGCGGGTACCCGCGGCCCGCGTGACGAACC		
SCO7378	SC10G8.05c	unknown	CGGTGTCGTCGGTACGGCGGCGGGTACCCGCCCGGCGAG		

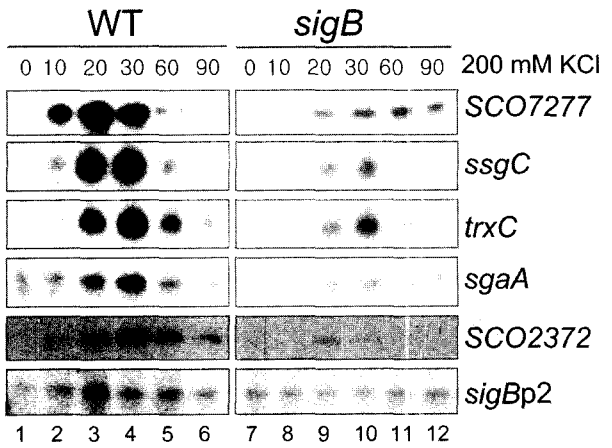

Fig. 1. σ^B-dependent induction of five candidate genes by KCl treatment as monitored by S1 mapping. Induction of *SCO7277* (encoding a putative anti-sigma factor of σ^L), *ssgC* (a sporulation protein), *trxC* (a thioredoxin), *sgaA* (suppressor of high osmolarity/A-factor growth defect), and *SCO2372* (a small hydrophobic protein) genes was monitored by S1 mapping following KCl treatment. *S. coelicolor* wild type (J1501 strain) and *sigB* mutant (YD2108) cells were grown in YEME liquid medium to early exponential phase and treated with 0.2 M KCl for 10, 20, 30, 60, and 90 min before harvesting cells. S1 mapping analysis was carried out using gene-specific probes, as described previously (Lee, 2003). As a control, constitutive *sigB*p2 transcripts were analyzed.

tions in spacer length, we also searched the genome for the consensus sequence pattern with a 14 base-pair spacer and found 43 additional candidate promoters. A total of 118 promoters upstream of 115 genes were listed and categorized by known and predicted function of downstream ORFs (Table 1).

This list displays putative σ^B-dependent genes with a variety of functions including stress response, transcriptional regulators, signal transduction, and so on. Many genes encoding sigma factors, and their regulators, were listed. These include σ^B and σ^L (SCO7278, a σ^B-like sigma factor), an anti-sigma factor of σ^H (PrsH), a putative anti-sigma factor of σ^L (SCO7277), three putative anti-anti-sigma factors of the RsbV family (SCO0869, SCO3067, SCO7325), and an ECF sigma factor (SCO5934). Quite a number of genes encoding membrane-associated, secreted, and cell-wall-related proteins were listed, suggesting the involvement of these gene products in osmotic protection and differentiation. Several differentiation-related genes were found, such as *catB*, *redZ*, *ssgC*, and *sgaA* encoding catalase, a specific activator for production of red antibiotic undecylprodigiosin, an SsgA-like sporulation protein, and an A-factor related protein, respectively (Ando *et al.*, 1997).

Among the listed genes, *sigB*p1, *catB*, *prsH*, *arsI* (*SCO3067*), *sigL* (*SCO7278*), and *rsbV* (*SCO7325*) have been induced by osmotic shock in a σ^B-dependent manner in *S. coelicolor* (Cho *et al.*, 2000, 2001; Lee, 2003). To estimate the validity of the *in silico* analysis, we tested the dependence of 5 candidate promoters on σ^B by S1 nuclease mapping (Fig. 1). The *sigB* mutant was generated from wild type J1501 cell by inserting a thiostrepton resistance cassette, as described in Cho *et al.* (2001). RNA samples were prepared from cells before and after treatment with 0.2 M KCl for 10, 20, 30, 60, and 90 min. S1 mapping analysis of transcripts from *SCO7277* (encoding a putative anti-sigma factor for σ^L), *ssgC*, *trxC* (encoding a thioredoxin), *sgaA*, and *SCO2372* (encoding a small hydrophobic protein) genes revealed that their osmotic induction is heavily dependent on σ^B. Constitutive expression from the *sigB*p2 promoter was presented for comparison. The induction kinetics of each gene is somewhat different, *SCO7277* being induced most rapidly within 10 min, and the rest after 20 min. The duration of induction was different as well. For *SCO7277* and *ssgC* the amount of transcripts returned to the pre-stimulus level within an hour, whereas it took longer for *trxC*, *sgaA*, and *SCO2372*. *SCO2372* maintained its induced level even 90 min after treatment. The observed differences in induction kinetics profile, as well as some residual induction in the *sigB* mutant, suggest that the osmotic induction of these genes might be dependent on σ^B and additional factors. The effect of σ^B on these genes could be either direct or indirect. The rapid and transient induction of SCO7277 could be due to direct control by σ^B, whereas the delayed induction of SCO2372 may be due to indirect control. Additional regulators may include some other σ^B-like sigma factors that share promoter consensus with σ^B and/or those regulators whose expression is controlled by σ^B. In *B. subtilis*, the σ^B-dependent salt induction of the σ^W regulon is well documented (Huang *et al.*, 1998).

For most regulons controlled by σ factors, consensus search procedures have not been particularly useful and sometimes even defining a consensus sequence can be difficult. However, this approach was successfully applied to characterize the σ^R regulon of *S. coelicolor* and σ^B regulon of *B. subtilis* (Petersohn *et al.*, 1999; Paget *et al.*, 2001). A prerequisite for this approach is knowledge of the promoter sequence recognized by the σ factor in question. In the case of the *S. coelicolor* σ^B, the presence of an autoregulatory site (*sigB*p1) upstream of the *sigB* gene provided a very useful starting point. Obviously, a significant disadvantage of this procedure is that many sites in the genome match consensus, but are apparently silent. This implies that additional sequence information is also important to determine promoter activity. It has been proposed previously that these additional sequence elements likely include the presence of AT-rich sequences upstream of -35 (UP elements), and a T-rich sequence just downstream of the -35 element (Huang *et al.*, 1999).

Experimental confirmation of expression patterns for each candidate gene is required, using more high-through-

put approaches such as microarray and quantitative RT-PCR analyses. At present, our *in silico* screening of possible σ^B regulon appears quite effective, and will serve as a good starting ground to elucidate the regulon. Since σ^B-like sigma factors could recognize similar promoter sequences as suggested for σ^H (Viollier *et al.*, 2003), further experimental verification of promoters recognized directly by σ^B is necessary. An improvement of σ^B consensus promoter sequence, generated from those experimentally verified promoters, will also allow distinction from sequences recognized by other σ^B-like sigma factors.

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