Published in final edited form as:

Mol Microbiol. 2012 July ; 85(2): 326–344. doi:10.1111/j.1365-2958.2012.08115.x.

Conservation of thiol-oxidative stress responses regulated by SigR orthologues in actinomycetes

Min-Sik Kim^{1,§}, Yann S. Dufour^{2,3,†,§}, Ji Sun Yoo¹, Yoo-Bok Cho¹, Joo-Hong Park¹, Gi-Baeg Nam¹, Hae Min Kim¹, Kang-Lok Lee¹, Timothy J. Donohue², and Jung-Hye Roe^{1,*}

¹Laboratory of Molecular Microbiology, School of Biological Sciences, and Institute of Microbiology, Seoul National University, Seoul 151-742, Korea

²Department of Bacteriology, University of Wisconsin, Madison, WI 53706, USA

³BACTER Institute, University of Wisconsin, Madison, WI 53706, USA

Summary

Numerous thiol-reactive compounds cause oxidative stress where cells counteract by activation of survival strategies regulated by thiol-based sensors. In *Streptomyces coelicolor*, a model actinomycete, a sigma/antisigma pair SigR/RsrA controls the response to thiol-oxidative stress. To unravel its full physiological functions, chromatin immuno-precipitation combined with sequence and transcript analyses were employed to identify 108 SigR target genes in *S. coelicolor* and to predict orthologous regulons across actinomycetes. In addition to reported genes for thiol homeostasis, protein degradation and ribosome modulation, 64 additional operons were identified suggesting new functions of this global regulator. We demonstrate that SigR maintains the level and activity of the housekeeping sigma factor HrdB during thiol-oxidative stress, a novel strategy for stress responses. We also found that SigR defends cells against UV and thiol-reactive damages, in which repair UvrA takes a part. Using a refined SigR-binding sequence model, SigR orthologues and their targets were predicted in 42 actinomycetes. This revealed a conserved core set of SigR targets to function for thiol homeostasis, protein quality control, possible modulation of transcription and translation, flavin-mediated redox reactions, and Fe-S delivery. The composition of the SigR regulon reveals a robust conserved physiological mechanism to deal with thiol-oxidative stress from bacteria to human.

Introduction

Cells are exposed to various forms of oxidative stressors such as reactive oxygen species (ROS), natural or xenobiotic redox-active compounds, or some antibiotics, which elicit the formation of ROS inside the cell. The response mechanism involves various enzymes to remove such oxidants, systems to repair and recycle damaged cell components, and to maintain optimal cell physiology (Imlay, 2008; Zuber, 2009). One form of oxidative damage that frequently occurs in proteins and small molecules is the oxidation of cysteine thiols by

*For correspondence. jhroe@snu.ac.kr; Tel. (+82) 2 880 6706; Fax (+82) 2 882 6706.

†Present address: Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.

§These two authors contributed equally.

ROS (Kiley and Storz, 2004; Jacob *et al.*, 2006). Cysteine thiols can also be modified by reactive nitrogen species (RNS) and thiol-reactive electrophiles (Hess *et al.*, 2005; Rudolph and Freeman, 2009)

The presence of ROS, RNS and other thiol-reactive compounds can be sensed directly through thiol-based sensor-regulators, which modulate the expression of genes encoding functions that constitute the biological stress response (Antelmann and Helmann, 2011). The best studied examples of thiol-based sensor-regulators that respond to ROS in bacteria include H₂O₂-sensing OxyR in *Escherichia coli*, organic peroxide-sensing OhrR in *Bacillus subtilis*, and the anti-sigma factor RsrA that senses thiol oxidation and modulates regulator SigR activity in *Streptomyces coelicolor* (Paget and Buttner, 2003; D'Autreaux and Toledano, 2007; Antelmann and Helmann, 2011). In eukaryotes, thiol-based redox switches have been well exemplified in Yap1 of *Saccharomyces cerevisiae* and the mammalian Nrf2/Keap1 system (D'Autreaux and Toledano, 2007; Kensler *et al.*, 2007; Brandes *et al.*, 2009). Representative target genes of these thiol-based regulators are those that encode thiol homeostasis system such as thioredoxin (Trx), glutaredoxin (Grx) and small molecular thiol systems (regulated by OxyR, SigR/RsrA, Yap1, Nrf2/Keap1), catalases and peroxidases (by OxyR and Yap1), organic hydroperoxidase (by OhrR), detoxification of electrophiles (by SigR and Nrf2), and some proteolytic system (by OxyR, SigR, Yap1, Nrf2) (Kensler *et al.*, 2007; den Hengst and Buttner, 2008; Imlay, 2008; Antelmann and Helmann, 2011).

A zinc-containing anti-sigma (ZAS) factor RsrA that binds an ECF (group 4) sigma factor SigR in *S. coelicolor* responds to diamide-induced thiol oxidation by forming disulphide bonds, releasing SigR to transcribe its target genes (Kang *et al.*, 1999; Li *et al.*, 2003b; Bae *et al.*, 2004). In addition to thiol-oxidants, the presence of non-oxidative thiol-reactive compounds also induces expression of the SigR regulon, suggesting that RsrA may respond to these compounds either directly through thiolation of reactive cysteines or indirectly through changes in reduced thiol pools such as mycothiol, the functional equivalent in actinomycetes of glutathione (Newton *et al.*, 2008; Park and Roe, 2008). To date, around 44 SigR target genes have been identified based on promoter sequence similarity, S1 mapping and microarray expression profiling (Paget *et al.*, 1998; 2001; Park and Roe, 2008; Kallifidas *et al.*, 2010). The products of known SigR target genes include thiol-redox proteins such as thioredoxin systems (TrxBA, TrxC), the first enzyme in mycothiol synthesis (MshA), and a glutaredoxin-like protein called mycoredoxin (Mrx). They also include proteolytic components of protein quality control (PepN, SsrA, ClpP1P2, ClpX, ClpC, Lon), cysteine production (CysM), methionine reduction (MsrA, MsrB), guanine synthesis (GuaB), ribosome-associated function (RpmE, RelA), and electrophile detoxification (Mca) (Paget *et al.*, 2001; Park and Roe, 2008; Kallifidas *et al.*, 2010). Therefore, perturbation of intracellular thiols, as sensed through RsrA, induces expression of gene products that contributes to protein quality control, detoxification of thiol-conjugative xenobiotics, and modulation of transcription and translation.

Because SigR/RsrA activity controls the cellular response to a variety of stressors, we expect the SigR regulon to be large and encompass additional stress response functions in *S. coelicolor*. Therefore, we performed chromatin immuno-precipitation on a chip (ChIP-chip) assays and discovered > 100 direct SigR target promoters transcribing > 160 genes. The

comprehensive characterization of the *S. coelicolor* SigR regulon helped us understand in more details the global effects of and cellular response to thiol-oxidative stress. Furthermore, to identify functions that are most critical to survive thiol-oxidative stress response, we used the SigR-binding consensus sequence to predict orthologous thiol-oxidative stress regulons in other actinomycetes. The analysis revealed a core conserved regulon as well as lineage-specific adaptations.

Results and discussion

Identification of SigR target genes by chromatin immuno-precipitation on a chip (ChIP-chip) and sequence analysis

To identify SigR target sites in the *S. coelicolor* genome we performed a ChIP-chip experiment using anti-SigR serum with wild-type cell cultures treated with diamide to activate SigR activity. We also performed the same ChIP-chip experiment with a *sigR* mutant to subtract non-specific chromatin enrichment because *S. coelicolor* possesses many additional sigma factors that may cross-react with the anti-SigR serum. After subtracting non-specific signal, we identified 122 regions significantly enriched by the immuno-precipitation of SigR (P -value = 0.05). Next, to identify more precisely putative SigR promoters, we searched within the enriched genomic regions for putative SigR promoters by detecting sequences that differed from the previously proposed SigR promoter (GGAAT-N₁₈-GTT; (Paget *et al.*, 2001) by at most 2 nucleotides with either of 2 possible spacer lengths (18 and 19 nucleotides) on either DNA strand. Only 5 of the 122 enriched regions did not contain at least one sequence matching our criteria, increasing the confidence that the regions enriched by the ChIP-chip assay are indeed SigR binding sites. Overall, 176 putative SigR promoter sequences were detected, and 108 of them are oriented towards annotated genes within 500 nt from the start codon.

In Table 1, we summarized genes predicted to be transcribed from these 108 promoters. Detailed information about the position of SigR-bound regions (enriched ChIP peaks), position and sequence of the predicted SigR promoters is presented in Table S1. Considering operon structures (<http://scocyc.streptomyces.org.uk>), more than 163 genes are predicted to be under the direct control of SigR. Among these, 44 promoters have been previously reported to produce SigR-dependent transcripts by S1 mapping experiments (Paget *et al.*, 2001; Park and Roe, 2008; Kallifidas *et al.*, 2010) or microarray studies (Kallifidas *et al.*, 2010). Re-examination of the transcriptional microarray data (Kallifidas *et al.*, 2010), using slightly different criteria (see *Experimental procedures*), predicts SigR-dependent transcripts from a total of 62 promoters within 57 of the regions bound by SigR *in vivo*. We marked these promoters in Table S1. Therefore, our ChIP-chip analysis revealed between 46 and 64 new SigR target promoters, undetectable from transcriptome profiling alone. The lack of detection in microarray analysis alone could be due partly to low level of gene expression and/or additional transcription from one or more SigR-independent promoter.

Supporting information

Additional supporting information may be found in the online version of this article.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

When 108 SigR target operons were classified on the basis of known and predicted functions, many of them fall in the functional groups related with maintenance of thiol redox homeostasis and sulphur-containing amino acids (11 operons; groups 1 and 2 in Table 1), protein folding and degradation (7 operons; group 5), and oxidoreductases (15 operons; group 8) (Table 1), fortifying previous proposals that the SigR regulon functions to achieve redox homeostasis and protein quality control under conditions of thiol-oxidative stress (Paget *et al.*, 2001; Park and Roe, 2008; Kallifidas *et al.*, 2010). Within these groups, we noticed newly added genes encoding a putative mycoredoxin (SCO3442), and a eukaryotic-type proteasome system that degrades proteins ligated with prokaryotic ubiquitin-like protein Pup (Mpa, PafD, Pup, PrcAB) first reported in *M. tuberculosis* (Pearce *et al.*, 2008; Burns and Darwin, 2010). This gene cluster encodes a putative AAA ATPase (Mpa), an accessory factor (PafD), prokaryotic ubiquitin-like protein (Pup), 20S proteasome alpha and beta subunits (PrcAB) similar to those encoded by *Mycobacterium tuberculosis* (Pearce *et al.*, 2008; Darwin, 2009). S1 mapping analysis demonstrates that *mpa* (SCO1648) has a SigR-dependent promoter, which is diamide-induced, in addition to ones that are independent of SigR (Fig. 1). Since the thiol-oxidant diamide can cause protein misfolding and aggregation (Kallifidas *et al.*, 2010), induction of proteolytic systems by diamide-triggered SigR matches biological necessity.

We also found a large number of SigR target genes that may function in modulating ribosome-associated processes (9 operons; group 3). Newly identified genes in this group encode probable translation initiation factors (IF-2, IF-3), 50S ribosomal proteins (L20, L35), ribosome-associated GTP-binding proteins (EngA, ObgE, Era), a ribosome binding factor RbfA, and a tRNA pseudouridine synthase (TruB). Observation of many ribosome-associated genes in the SigR regulon leads us to postulate that thiol-oxidative stress may somehow hinder translation processes, against which cells induce a SigR-mediated response. Induction of tmRNA (*ssrA*) by SigR under thiol-oxidative stress suggests an increase in truncated mRNA in the A-site of ribosome in the cell. It has been shown in *E. coli* that ribosome stalling induces A-site bound RelE or RelE-like RNA interferases to cut mRNA (Neubauer *et al.*, 2009; Christensen-Dalsgaard *et al.*, 2010), whose continued translation requires tmRNA that adds the SsrA tag to the truncated protein for degradation. In this respect, a recent discovery of the role of tmRNA in relieving polyribosomal stalling in *dnaK* and other stress-related mRNA to facilitate their translation in streptomycetes highlights a potential new link between ribosomal modulation and stress response (Barends *et al.*, 2010). Increased demand for guanine nucleotides due to elevation in activity of RelA and GTP-binding proteins during stress could be met by increased GMP synthesis by *guaB* and *guaBI* gene products. Increased demand for guanine nucleotide can also be explained by the fact that guanine is most vulnerable to oxidation among four DNA bases due to its lower oxidation-reduction potential (Kawanishi *et al.*, 2001).

Many transcription factors (15 operons; group 6) are predicted to be SigR targets, implying that SigR is a global regulator that can change the transcription profile through cascades of affected transcriptional regulators. Among newly identified SigR targets are those encoding the major housekeeping sigma factor HrdB (SCO5820) and a SigR-like ECF sigma factor SigR2 (SCO3450) and its putative partner RsrA2-2 (SCO3451). The gene for an IclR-like

transcription factor (SCO5552) called 'NdgR' that regulates amino-acid-dependent growth and antibiotic production (Yang *et al.*, 2009) also contains a SigR-binding promoter sequence.

Synthesis of a number of redox-sensitive cofactors (such as Fe-S, CoA, NAD, lipoate, folic acid, pyridoxal phosphate and molybdopterin) appears to be regulated by SigR (9 operons; group 11). In addition to the previously identified *sufA*, *coaE*, *folE* and *moeB* genes [encoding a putative Fe-S assembly factor, coenzyme A kinase, GTP cyclohydrolase I for folate biosynthesis, and sulphurylase possibly involved in molybdopterin synthesis (Paget *et al.*, 2001) respectively], we found that *sufU*, *nadA*, *lipA* and *pdxH2/3* [encoding putative NifU-type Fe-S assembly factor, quinolinate synthetase A subunit for nicotinamide synthesis, lipoic acid synthase, and phospho-pyridoxamine synthetase for pyridoxal phosphate (vitamin B6)] were all direct targets of SigR. It is interesting to note that some of these proteins are either putative Fe-S assembly components (SufA, SufU) or Fe-S containing enzymes (NadA, LipA). In addition, the cofactors they synthesize contain sulphur atoms (Fe-S, folate, CoA, lipoic acid), or involve sulphur-mediated reactions (LipA, MoeB). Exposed Fe-S cofactors are labile to oxidation, so we propose that increased SigR activity contributes to replenishing Fe-S cofactors, Fe-S-containing proteins, and/or other oxidation-labile S-containing factors upon oxidative stress.

S1 nuclease mapping confirms predicted SigR promoters

To provide additional experimental evidence for some of the newly identified SigR targets, we examined 25 selected transcripts by S1 nuclease mapping at different times after exposing *S. coelicolor* cells to the thiol oxidant diamide. We observed SigR-specific transcripts from 24 promoters whose 5' ends match with the predicted location of the promoters (Table S1). This supports the hypothesis that nearly all the SigR regulon genes presented in Table 1 produce SigR-dependent transcripts. We detected new SigR-dependent transcripts from 15 promoters whose expression has not been previously reported (SCO1084, 1600, 1648, 1758, 1936, 2194, 2254, 2595, 2763, 3296, 3442, 4797, 5705, 5820, 7784), confirming that ChIP-chip analysis detects SigR target genes with high sensitivity. Figure 1 shows S1 mapping analysis of transcripts from these promoters. Some genes produce transcripts initiated primarily from SigR-dependent promoters (Fig. 1A; 1084, 1920, 1936, 1958, 2161, 2194, 2537, 2763, 3091, 3373, 4956, 5705, 6061, 7632, 7784). Others utilize additional SigR-independent promoters (Fig. 1B; 1600, 1758, 2254, 2595, 3296, 4797).

Examination of genes with multiple promoters reveals diverse patterns of SigR-dependent modulation. For example, SCO1758 (*engA*) encoding a putative GTPase produces p1 transcript as predicted (250 nt upstream from the start codon; Table S1) and also the downstream unpredicted p2 transcripts, whose promoter sequence (GGAT-N16-GTT; **ggatcacccggtaaaggggtgtt**) resembles the SigR consensus but with a shorter spacing of 16 nucleotides. This implies a flexibility of spacing between -35 and -10 elements of SigR-dependent promoters. In some genes such as SCO2254 the dominant transcripts produced under non-stressed condition disappear transiently following stress independent of SigR, during which period the SigR-dependent transcripts are induced.

In promoters such as p2 of SCO1758 (*engA*; Fig. 1B) and SCO3442 (*mrxB*; Fig. 1A) (encoding a paralogue of mycoredoxin and a glutaredoxin-like protein) the SigR-induced transcripts still appear in the *sigR-rsrA* mutant but with delayed response. A similar phenomenon was also observed for SCO3091 (*cfa*) encoding a putative cyclopropane-fatty acyl phospholipid synthase (Fig. 1A). The delayed induction kinetics in *sigR-rsrA* mutant suggests that these SigR-dependent promoters may also be recognized by SigR paralogues that recognize similar promoter sequences as SigR. Similar induction pattern has been reported for transcripts encoding Lon protease, which are induced rapidly by SigR but with slower kinetics in the absence of SigR (Kallifidas *et al.*, 2010). SCO3450 and SCO3451, which are SigR regulated genes encoding homologues of SigR (SigR2) and RsrA (RsrA2-1), respectively, show a similar pattern of induction (data not shown). A likely candidate for a sigma factor that shares similar promoter recognition sequence with SigR is SCO5147 (SigR1), which is a close homologue of SigE from *M. tuberculosis* (MtbSigE) as exemplified in the phylogenetic comparison of ZAS-linked ECF sigma factors from *S. coelicolor* and *M. tuberculosis* (Fig. S2). The promoter for *sigR1* has GGAAC-N18-GTT sequence that is not bound by SigR in our ChIP analysis. It is induced by diamide with slower kinetics (data not shown), and we propose that it is recognized by SigR1 itself. These observations indicate that a subset of SigR regulon genes can be recognized by at least one closely related SigR paralogue in addition to or in the absence of SigR.

SigR maintains the level and activity of the housekeeping sigma factor HrdB during thiol-oxidative stress

The SigR regulon includes several known or predicted transcription factors (SCO1425, 1619, 2331, 2481, 2537, 3207, 3450, 5065, 5552, 6775, 7140), which may significantly increase the number of genes regulated in response to thiol oxidative stress. In addition, we found that the major housekeeping sigma factor HrdB (SCO5820) possesses a previously unrecognized SigR-dependent promoter (GGAAT-N18-GCT) about 50 nt downstream of the known SigR-independent promoter (p1) (Buttner *et al.*, 1990) (Fig. 2A). S1 mapping analysis of *hrdB* transcripts revealed that p1 is indeed the primary promoter in the absence of stress. However, diamide stress rapidly decreased the amount of p1 transcripts and increased the SigR-dependent transcripts from the predicted down-stream p2 promoter (Fig. 2B). The decrease in p1 transcripts upon oxidative stress occurred independently of SigR. This indicates that SigR functions to elevate *hrdB* transcription under oxidative stress conditions. Existence of a second promoter to compensate for loss of transcription from the primary promoter is also observed in SCO2254 encoding a putative efflux membrane protein (Fig. 1B), suggesting that this phenomenon may not be rare among SigR-regulated genes. Whereas some primary promoters such as p1 of SCO5820 (*hrdB*) and p1 of SCO2254 are affected dramatically by diamide treatment, the primary promoter p1 of SCO4797 (*uvrD*) is not affected at all. Therefore, the inhibitory effect of oxidative stress on transcription from a major p1 or housekeeping promoter may be a specific response at a subset of genes.

To test whether SigR controls the amount of HrdB protein during oxidative stress, as predicted from transcript analysis, we monitored the amount of HrdB protein by analytical immunoblot analysis. To ensure quantitative detection of HrdB, we diluted cell extract with

cell lysis buffer containing non-specific BSA protein as a protein buffer that could minimize uncontrolled loss of proteins in cell extract during liquid handling and electrophoresis. Results in Fig. 2C demonstrate that HrdB protein is maintained during oxidative stress condition at a level slightly higher than that under non-stressed condition in the wild type. On the contrary, the level of HrdB decreased significantly in *sigRrsrA* mutant upon oxidative stress condition. Even though it is not clear why the level of HrdB protein increases in the wild type during the stress, it is obvious that SigR does contribute to maintain the level of HrdB protein, as predicted from transcript analysis. We further monitored the activity of HrdB by examining transcripts from HrdB target promoters (*rrnD*p1, p3 and p4) in the ribosomal RNA operon *rrnD*, in the wild type and mutant. All four promoters of the *rrnD* operon share the consensus sequence for HrdB recognition, especially in the -10 region, to varying extent (Baylis and Bibb, 1988; Hahn and Roe, 2007). *In vitro* transcription from *rrnD* promoters with reconstituted holoenzyme containing HrdB ($E\sigma^{\text{HrdB}}$) has demonstrated that all four promoters can be transcribed by $E\sigma^{\text{HrdB}}$, even though transcription from p3 and p4 promoters requires additional cellular factors (Hahn and Roe, 2007). We found that the amounts of the abundant p3 and p4 transcripts were maintained throughout the stress period and even increased up to 50% and 30%, respectively, during 40–80 min diamide treatment. On the other hand, they both decreased continuously in the *sigRrsrA* mutant to about 40% and 60% level, respectively, during the 2 h of stress (Fig. S3). Transcripts from the p1 promoter decreased to 20% level during the stress in the mutant, whereas the level was maintained in the wild type (Fig. S3). These results clearly demonstrate that SigR is necessary to maintain the level and activity of the major housekeeping sigma factor during thiol-oxidative stresses.

The UvrA protein is necessary to cope with UV and thiol-oxidative stresses

Among the newly discovered SigR targets are SCO1958, SCO4797 and SCO5188, which encode putative UvrA, UvrD and a paralogue of UvrD (UvrD2), respectively, predicted to function in nucleotide excision repair. The UvrABC endonuclease complex with UvrD (DNA helicase II) has been shown to repair UV-induced DNA damages, the most prominent of which are pyrimidine dimers (Sinha and Hader, 2002). However, whether the UvrABC system is needed under thiol-oxidative stress condition and whether thiol-oxidative stress causes DNA damage have not been known. Consensus SigR promoters (GGCAT-N18-GTT for *uvrA*, GGCAT-N18-GTT for *uvrD*, GGAAA-N19-GTC for *uvrD2*) are located 103, 31 and 7 nt upstream from the start codons of respective genes (Table S1). The *uvrA* gene is transcribed at a very low level under unstressed conditions. However, upon diamide stress, it is drastically induced from a SigR-dependent promoter (Fig. 1A). On the contrary, the *uvrD* gene is transcribed at a relatively high level from a SigR-independent promoter p1 during unstressed condition. Upon induction, the upstream SigR-dependent promoter p2 is induced (Fig. 1B). The *uvrD2* transcripts were not detectable by S1 analysis, probably due to low amount.

In order to examine whether UvrA indeed confers UV resistance, and whether SigR and UvrA have related functions in conferring resistance to UV and thiol-reactive compounds in *S. coelicolor*, we made a *uvrA* strain and analysed its sensitivity to UV and thiol-reactive compounds along with *sigR-rsrA* mutant. Figure 3 demonstrates that *uvrA* has increased

sensitivity to UV irradiation, and *sigR-rsrA* is also sensitive to UV, though not as dramatically as *uvrA*. Therefore, SigR is necessary to achieve optimal UV resistance possibly through inducing UvrA system. The SigR regulon genes were not induced by UV irradiation (data not shown), suggesting that UV itself is not capable of activating SigR function. SigR can be activated by thiol-reactive oxidants/electrophiles such as diamide and methyl hydroquinone (MHQ), as predicted from the induction of thiol-specific oxidative stress response by these compounds in *B. subtilis* (Antelmann *et al.*, 2008; Nguyen *et al.*, 2009). Since some electrophiles can react not only with thiols in proteins and small molecules but also with DNA, we examined whether UvrA is needed to cope with chemical stresses caused by thiol-reactive electrophiles such as diamide, methyl hydroquinone (MHQ) and methyl glyoxal (MG). It has been reported that MG induces not only thiol-specific oxidative stress response but also SOS response, suggesting some DNA damage in *B. subtilis*. (Nguyen *et al.*, 2009). MG is known to cause DNA damage by reacting with DNA bases to form *N*²-(1-carboxylethyl)-2'-deoxyguanosine (CEdG) (Synold *et al.*, 2008). Whether this kind of damage is repaired by the Uvr system has not been reported. Whether thiol-reactive diamide and MHQ could elicit DNA damage has not been reported either. The result in Fig. 3 shows that UvrA is needed to defend cells against these electrophiles. As expected, *sigR-rsrA* mutant became very sensitive to thiol-reactive electrophiles. Dramatic resistance of *sigR-rsrA* toward MG is unexpected. Inability to induce many thiol-homeostatic components may somehow confer better survival relative to the wild type in the presence of MG in plate culture. When the sensitivity of *sigR-rsrA* toward MG was monitored in liquid culture during early exponential phase (OD ~ 0.3), the mutant was more sensitive to MG than the wild type, suggesting that the resistance phenotype on plate culture could depend on treatment method (data not shown). Overall, the finding that SigR induces DNA repair proteins expands our understanding of the function of SigR to accommodate response towards UV- and some electrophile-incurred damages.

The phylogenetic analysis of SigR orthologues and their predicted respective regulons reveals a core conserved thiol oxidative stress regulon in *Actinomycetes*

The characterization of this larger SigR regulon in *S. coelicolor* furthers our understanding of the organism's response to thiol oxidative stress. However, it has been previously observed that the composition of a given regulon in a given organism may result from specific adaptations to its ecological niche (Dufour *et al.*, 2008; 2010a; Perez and Groisman, 2009). Therefore, the SigR regulon in *S. coelicolor* may comprise many functions that are specific only to this organism, and not shared by other organisms. Therefore, comparison of predicted SigR regulons in other organisms may indicate functions that are commonly required to cope with thiol oxidative stress in general apart from those specifically required for this organism. To test this hypothesis and predict which genes found in the *S. coelicolor* SigR regulon are most likely to be directly relevant to thiol oxidative stress, we performed a phylogenetic analysis of SigR-RsrA orthologues and their regulons across a group of *Actinomycetes* using a computational method previously described (Dufour *et al.*, 2008; 2010a). Since this phylogenetic analysis relies on the accurate computational detection of putative SigR promoter sequences, we constructed a refined SigR promoter model by aligning the sequences of predicted promoters corresponding to 57 enriched regions in the ChIP-chip assay that matches with genes transcribed in a SigR-dependent manner (Table

S1). Figure 4A demonstrates a new SigR consensus logo, with a representative sequence of GGAAT-N18-19-GTT.

We first detected and aligned the protein sequences of all SigR orthologues found in 42 *Actinomycetes* to determine the conservation of their DNA binding domains (only *Mycobacterium leprae* TN did not possess SigR and RsrA orthologues). The multiple sequence alignment showed that the protein domains determining promoter sequence specificity (regions $\sigma 2.4$ and $\sigma 4.2$) of SigR orthologues are well conserved across all species (Fig. S1). Therefore, we assumed that all SigR orthologues recognize very similar if not identical promoter sequences. Thus, we identified putative SigR targets across all the selected genomes by searching in each genome for sequences matching *S. coelicolor* SigR promoter model (Fig. 4A) in the upstream of annotated genes. With the promoter score cut-off chosen for this analysis, we captured 72 of the 108 SigR promoters defined by our ChIP-chip analysis in *S. coelicolor*. Finally, putative SigR target genes across the 42 genomes were grouped by orthology and clustered according to the occurrence of predicted SigR-dependent promoters.

The emerging phylogenetic patterns predicted that the top 25 sets of orthologous genes appear to be regulated by SigR orthologues across most of the 42 *Actinomycetes*, thus, forming a core conserved regulon for the response to thiol oxidative stress (Fig. 4B, Table 2). The predictions resulting from this comparative genomics analysis are well supported by the experimental data presented in this report because 20 of the *S. coelicolor* genes corresponding to the top 25 conserved SigR targets are associated with SigR ChIP-chip peaks (Table 2). Excluding the regulator pair SigR-RsrA, the predicted functions of these genes fall in 6 categories: (i) thiol redox homeostasis with *trxB* (#80), *trxC* (#217), *mrxA* (#927), *msrA* (#157) and 2 Dsb-like proteins (#1014, #1783); (ii) protein quality control with *dnaK* (#61), *grpE* (#445), *clgR* (#646), *clpC* (#1) and *htpX* (#723); (iii) flavin-mediated oxidoreductases (#838, #1779, #1973); (iv) translational modulation with *prfA* (#236) and *rpmE1* (#37); (v) transcriptional modulation with *rbpA* (#598) and *Mtb-sigB* (#2534); (vi) iron-sulphur cluster assembly with *sufA* (#677). Additionally, hypothetical proteins of unpredictable functions (#852, #1035, #1997, #5796) were included. Table 2 summarizes the description of each set of orthologous genes, by presenting its representative gene and the genera in which it occurs among 22 genera examined. The analysis predicts that the thiol redox homeostasis and protein quality control are the two most outstanding functions in the core SigR regulon across actinomycetes, followed by transcriptional and translational modulations. *Mtb-sigB*, a group 2 sigma factor, controls general stress response and is induced by a variety of stressors and by multiple sigma factors such as SigH and SigE in *Mycobacterium tuberculosis* (Sachdeva *et al.*, 2010). Therefore, in many actinomycetes, it is likely that SigR orthologues control the expression of another general stress factor of non-ECF family, suggesting that the stress signal initiates a transcriptional cascade, which includes multiple DNA binding proteins. The function of RbpA is not well understood except that it associates with RNA polymerase and confers resistance to rifampicin in actinomycetes (Newell *et al.*, 2006; Dey *et al.*, 2010). PrfA terminates translation as a peptide release factor and recycles ribosome whereas RpmE1 is the ribosomal protein L31. How the induction of genes for PrfA and RpmE1 is beneficial under thiol-oxidative

conditions is not clear. The *rpmE1* gene encoding an isoform of L31 with cysteine residues for zinc-binding is induced by SigR, whereas the cysteine-negative paralogue *rpmE2* is induced by Zur under zinc-depleted condition in *S. coelicolor* (Owen *et al.*, 2007; Shin *et al.*, 2007). Whether thiol oxidations in ribosomal components or translational complex causes increased demand for PrfA and RpmE1 and whether zinc-containing RpmE1 is sensitive to oxidative stress deserve future studies.

When we extended the analysis to include less conserved genes up to 250 gene sets, we found that some gene sets are still conserved significantly across actinomycetes, whereas others are more restricted to certain genera. Figure 5 displays the clustered pattern of these gene sets to exhibit a relatively common group (E) and genus-specific groups (A, B, C, D, F, G), distributed to a specific genus such as *Streptomyces* (group A&E), *Salinispora* (group B&E), *Frankia* (group C&E), *Corynebacterium* (group D), *Mycobacterium* (group E&F) and *Arthrobacter* (group G). The so-called extended regulon of *S. coelicolor* (group A&E) contains 79 genes, and among genes with predicted functions the main functions represented are: electron transport (9 genes), biosynthesis of cofactors (9 genes), transcriptional regulators (6 genes), and protein homeostasis (5 genes). However, only 29 of these 79 putative targets are associated with a ChIP-chip peak from our analysis, suggesting that the computational detection of SigR targets that are not broadly conserved across the phylogeny may be impaired by a high rate of false-positive in this analysis (Fig. 5, Table S2).

SigR and its paralogues with shared promoter selectivity – Overlap of SigR regulon with other regulons

Among the top 25 conserved SigR regulon members, *dnaK* (#61) and *grpE*(#445) are not directly dependent on SigR in *S. coelicolor*. The consensus SigR logo is not found upstream of the *dnaK-grpE-dnaJ-hspR* operon in *S. coelicolor* as well as in all the other *Streptomyces* genomes examined in this study. In *M. tuberculosis*, transcription of the *dnaK* operon is activated in response to heat shock and oxidative stress by SigE, a paralogue of SigH (SigR orthologue) (Fig. S2), with similar recognition sequence (Song *et al.*, 2008). Mtb-*sigB* gene (#2534) is also recognized by SigE in *M. tuberculosis*. Since Mtb-SigH induces the expression of the *sigE* gene (Raman *et al.*, 2001; Manganelli *et al.*, 2002), there exist two tiers of overlap between regulons of SigH and SigE in *M. tuberculosis*, arising from similar recognition sequence and from hierarchically sequential induction. Likewise, it is conceivable that the *dnaK* operon in *S. coelicolor* is regulated by a SigR paralogue that resembles Mtb-SigE. If this were true, SigR1 (SCO5147) is the most likely candidate for activating the *dnaK* operon in *S. coelicolor* (Fig. S2). SCO5147 could play a similar role in other species since it is found among the top 50 conserved sets of SigR-regulon members in actinomycetes (Table S2, group E). In *M. tuberculosis*, the SigE-binding consensus (GGAACY-N17-18-GTT) overlaps significantly with SigH (SigR orthologue)-binding consensus (GGAAYA-N17-18-GTT) (Sachdeva *et al.*, 2010). It has been reported that a single position in the -35 element primarily distinguish the recognition specificity of SigE from that of SigH (Song *et al.*, 2008). Considering this, it is likely that the list of bioinformatically predicted SigR regulon genes includes not only those directly recognized by SigR orthologues, but also some that are recognized by SigE-type paralogues. In addition, since *sigE* transcription requires SigH in *M. tuberculosis*, the predicted SigR/SigH

regulon members may include indirectly regulated ones due to regulator cascade. The extent of overlap between SigR orthologues and their close paralogues in sequence specificity as well as hierarchical regulation may differ in different organisms. Experimental verification may require not only the comparison of transcripts between the wild-type and regulator mutants, but also regulator binding studies *in vivo* and *in vitro*.

Involvement of ClgR in the core SigR regulon is intriguing (Table 2). The *clgR* gene (SCO5755) is co-transcribed with SCO5754, a SigR target gene which encodes a homologue of CinA known to be induced by DNA damage and competence in *Streptococcus pneumoniae* (Martin *et al.*, 1995). In *S. coelicolor* ClgR positively regulates genes for proteases (*clpP1/P2*, *lon*, *clpC*) that are direct targets of SigR (Bellier and Mazodier, 2004). In *C. glutamicum*, ClgR controls transcription of genes involved in proteolysis and DNA repair such as *ptrB* encoding endopeptidase, *hflX* encoding a GTP-binding protein, *recR* for recombinational repair of UV-induced DNA damage (Engels *et al.*, 2004). Inclusion of known ClgR targets in the SigR regulon of *S. coelicolor* demonstrates an interesting regulatory circuit that involves double positive regulators. The contribution of SigR appears critical to allow promoter recognition as well as provision of ClgR in transcribing ClgR target genes.

Functional overlap between prokaryotic SigR regulon and eukaryotic Nrf2 regulon suggests a broad conservation of the thiol oxidative stress response

Examination of SigR regulon genes in *S. coelicolor* reveals a striking functional similarity with an analogous system in mammals as summarized in Fig. 6. The Nrf2-Keap1 system in higher eukaryotes constitutes a defence mechanism against stresses imposed by electrophiles and oxidants. Nrf2 is associated with its inhibitor Keap1, which facilitates ubiquitination and proteosomal degradation of Nrf2. Attacked by electrophiles or oxidants, Nrf2-Keap1 interaction is disrupted, and the released Nrf2 induces its regulon genes through binding to antioxidant response elements (ARE) (Kensler *et al.*, 2007). Nrf2 protein itself does not have any sequence homology with SigR. However, the mechanism by which it is activated involves thiol-disulphide isomerization of its inhibitor Keap1 as occurs in the activity modulation of SigR by RsrA (Dinkova-Kostova *et al.*, 2002). Similarly to RsrA, Keap1 has many cysteine residues (27 cysteines) and responds to oxidative and electrophilic stressors. Functions of the downstream target genes also overlap significantly between the SigR/RsrA and Nrf2-Keap1 systems. When activated, Nrf2 induces genes for various thiol homeostatic proteins, such as glutathione peroxidase, γ -glutamyl-cystein synthetase, thioredoxin reductase and peroxiredoxin 1, which are functionally related with thiol redox proteins, such as thioredoxins and their reductase, peroxiredoxin, mycothiol synthetic enzymes, and mycoredoxins regulated by SigR (Thimmulappa *et al.*, 2002; Rangasamy *et al.*, 2004). Just as Nrf2 increases synthesis of proteasome subunits and γ -glutamyl peptidase, SigR activates expression of *lon*, *pepN*, *clp* proteases and *mpa* (Kwak *et al.*, 2003). The amino acid sequence of *pepN* is homologous to γ -glutamyl peptidase. Both Nrf2 and SigR regulate pentose phosphate pathway enzymes such as glucose 6-phosphate dehydrogenase and transaldolase for generating reducing potential NADPH. The SCO2763 gene product, a putative ATP-binding cassette (ABC) transporter, is a close homologue of multidrug resistance-associated protein 1 (Mrp1/Abcc1) whose expression depends on Nrf2 and is

responsible for cellular extrusion of GSH-conjugates (Hayashi *et al.*, 2003). Whether SCO2763 has a function in exporting mycothiol (MSH)-S-conjugates of electrophiles deserves experimental study.

Conclusions

Genome-wide identification of direct SigR binding sites under thiol-oxidative stress conditions, combined with transcript and sequence analyses, revealed many new genes of SigR regulon in *S. coelicolor*. The wide spectrum of target genes that include cascades of regulators indicates that SigR is a global regulator. As expected, SigR induces many genes related with homeostasis of thiols in proteins and small molecules (mycothiols), and proper protein quality control, which are most prominently affected by thiol-reactive/oxidative stressors. Additionally, SigR induces many genes for ribosome-associated components, transcriptional regulators including sigma factors, numerous oxidoreductases (many of which are predicted to contain flavin), DNA damage repair, cofactor metabolism that include several Fe-S containing enzymes/proteins, and lipid synthesis. The full list of SigR target genes enabled prediction and experimental demonstration of new physiological functions of the SigR regulon under thiol-oxidative stress condition. We found that SigR directly contributes to maintain the level and activity of the major housekeeping sigma factor HrdB through increasing its transcription during oxidative stress. SigR also confers resistance to UV and thiol-reactive compounds, likely by inducing enzymes of DNA damage repair. Generation of a refined SigR promoter model enabled comparative genomics analysis of the SigR regulon, which revealed the existence of a core SigR regulon conserved across 42 selected *Actinomycetes*. The most prominent functions of the conserved SigR-orthologue regulons are related with thiol redox homeostasis, including the maintenance of sulphur-containing amino acids, and protein quality control involving chaperones and proteolytic systems. In addition, genes for translational modulation, transcriptional regulatory cascades, oxidoreductases and Fe-S assembly functions are also included. The bioinformatic analysis also predicts the existence of variable extended regulons specific for each genus. These results complement the experimental characterization of the SigR regulon in *S. coelicolor* to identify genes and functions that are most likely to be critical for the biological response to thiol oxidative stress. Comparison of SigR-regulated gene functions with those of thiol-oxidative stress response system (Nrf2-Keap1) in mammals reveals many shared gene functions, suggesting a robust physiological mechanism to deal with thiol-reactive stresses across distantly related life forms.

Experimental procedures

Strains and media

Spores of *S. coelicolor* A3(2) strain M145 were inoculated in YEME liquid medium containing 5 mM MgCl₂•6H₂O and 10% sucrose, and were grown at 30°C (Kieser *et al.*, 2000). NA plates (0.8% nutrient broth, 2% agar powder) were used for spotting test. *E. coli* was grown in LB broth (1% tryptone, 0.5% yeast extract and 1% NaCl). *E. coli* ET12567, a non-methylating strain containing pUZ8002 for donor functions and BW25113 harbouring

pIJ790 for linear DNA recombination were used for disruption as recommended (Gust *et al.*, 2002).

Chromatin immunoprecipitation on a chip (ChIP-chip) analysis

The probes used to fabricate the custom-made 385 K NimbleGen microarray (Roche NimbleGen, Madison WI) for the ChIP-chip experiment were designed using the ‘chipD’ software (Dufour *et al.*, 2010b). The parameters were set to obtain complete tiling of the *S. coelicolor* A3(2) genome with overlapping isothermal probes that ranged from 35 to 75 bases with an average spacing of 24 bases. In addition, probes alternate between the forward and reverse orientation to capture both DNA strands. The probe sequences and the ChIP-chip data are available at the NCBI’s Gene Expression Omnibus database with the accession number GPL14825 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL14825>).

Streptomyces coelicolor cells (wild-type M145 and *sigRrsrA* mutant strains) were grown to exponential phase (OD ~ 0.3) and were treated with 0.5 mM diamide for 20 min to prepare samples for the ChIP-chip experiment. The samples were prepared as previously described (Dufour *et al.*, 2008). Briefly, the cell cultures were fixed with 1% formaldehyde for 4 min at 30°C followed by quenching with 0.125 M glycine for 30 min in ice, washed with phosphate-buffered saline, and frozen. Cells were suspended in 100 mM Tris (pH 8.0), 300 mM NaCl, 2% Triton X-100, and 1 mM phenylmethylsulphonyl fluoride, and sonicated to lyse cells and fragment the genomic DNA. Micrococcal nuclease (100 U ml⁻¹) and RNase A (1 µg ml⁻¹) were added to the preparation to further fragment the genomic DNA and degrade RNA. Cell debris was removed by centrifugation and an aliquot was removed to analyse DNA fragmentation by agarose gel electrophoresis (desired size of ~200–1000 bp with enrichment for ~500 bp molecules). Anti-SigR rabbit polyclonal serum (7.5 µl) was used to immunoprecipitate cross-linked DNA fragments for each sample prepared from about 50 ml culture. A mock immunoprecipitation was performed on the *sigRrsrA* cells using the same serum to detect non-specific interactions. The enriched DNA was amplified via ligation-mediated PCR amplification using oligonucleotide linkers (oJW102: GCGGTGACCCGGGAGATCTGAATTC; oJW103: GAATTCAGATC). The DNA enriched using anti-SigR serum was labelled with Cy5 dye while DNA from the mock precipitation was labelled with Cy3. Labelled DNA from three independent immunoprecipitation experiments were pooled together in equal proportion to obtain the 4 µg of DNA that was hybridized on a microarray. Sample hybridization and microarray scanning were performed by Roche NimbleGen (Madison, WI) according to their standard protocol.

For each array, the Cy3 and Cy5 signals were normalized using the *vsn* method (Huber *et al.*, 2002) in the *R* statistical software environment before calculating the log₂ ratio of the experimental signal (Cy5) over the control signal (Cy3). A plot of the intensity ratios versus intensity averages revealed no dye bias. Therefore, no further correction was applied to the data. To remove the signal generated by non-specific DNA immuno-precipitation, the signal from the *sigRrsrA* cells was subtracted from the signal obtained from the wild-type cells. Only one array was hybridized for each *S. coelicolor* strain. Regions of the genome enriched for occupancy by SigR were detected using the TAMALPAIS method (Bieda *et al.*, 2006) at *P*-value 0.05 using a threshold set at the 99th percentile of the log₂ ratio. The relative peak

intensities were calculated by averaging the 10 highest consecutive probe signals for each selected peak.

Re-examination of expression profiling experiments under diamide stress

The microarray data of Kallifidas *et al.* (2010) were re-examined. To increase the detection sensitivity of SigR-dependent transcripts, the change in transcript abundances in the wild-type strain (M600) was divided by the change in transcript abundances in the *sigR* mutant strain (J2139) measured 20 min after the addition of diamide. Transcripts that were at least 1.5-fold more abundant in the wild-type strain were selected to identify putative members of the SigR regulon. Taking the ratio of the changes in abundance may help identify SigR-dependent transcripts that did not show net increase in abundances in the wild-type strain because higher activity of the SigR-dependent promoter may be accompanied by lower activity of an alternative promoter.

S1 nuclease mapping

Cells were treated with 0.1 ~ 0.5 mM diamide at the exponential growth phase (OD ~ 0.3) for 20–120 min. After harvest, they were disrupted by sonication in Kirby mix. RNA isolation and S1 nuclease mapping was performed according to a standard procedure (Kieser *et al.*, 2000). Promoter regions (from –500 to +60 nt relative to each start codon of SigR regulon gene) were amplified by PCR and used as probes. Probes were labelled with [γ -³²P]-ATP and hybridized with 5–50 μ g RNA at 50°C. After S1 nuclease treatment, protected DNAs were separated on 5% polyacrylamide gel containing 7 M urea. Signals were detected and quantified by BAS-2500 (Fuji film).

Western blot analysis

Cells were grown and treated with diamide as described for sample preparation for S1 mapping. Harvested cells were re-suspended in lysis buffer [20 mM Tris-HCl (pH 7.9), 10% (v/v) glycerol, 5 mM EDTA, 0.1 mM DTT, 10 mM MgCl₂, 1 mM PMSF, 0.15 M NaCl]. The suspension was sonicated with ultrasonicator (Sonics and Materials), and cleared by centrifugation. Protein concentration in crude cell extract was determined by Bradford reagent solution (Bio-Rad) using BSA as a standard. For each sample, ~ 10 μ l cell extract containing 50 μ g protein was further diluted to the final concentration of 0.125 μ g μ l⁻¹ with lysis buffer (up to 400 μ l) that additionally contain 50 μ g BSA to serve as a protein buffer. Aliquots of 8 μ l containing 1 μ g crude protein extract and the same amount of BSA were resolved on 10% SDS-PAGE. Immuno-detection by polyclonal mouse antibody against HrdB protein and the anti-mouse IgG secondary antibody at 1:5000 dilution ratio, followed by ECL detection system (Amersham Life Science), was done as described previously (Kim *et al.*, 2009).

Sensitivity test

The disruption mutant of SCO1958 (*uvrA*) was obtained by using PCR-targeting mutagenesis protocol (Gust *et al.*, 2002) as described previously (Kim *et al.*, 2009). The wild-type and mutant spores (10⁵, 10⁴, 10³) were spotted on NA media. For UV sensitivity assay, the spotted spores were exposed to UV in the CL-1000 UV cross-linker at 50 or 100 J

m⁻² before incubating. For chemical sensitivity, spores were spotted on NA media containing 0.6 mM diamide, 1 mM methylhydroquinone or 1 mM methylglyoxal, and incubated from 2 to 6 days at 30°C.

Determination of orthologous genes and phylogenetic tree reconstruction

All the genome sequences were obtained from the Integrated Microbial genome database on September 2008 (<http://img.jgi.doe.gov>) (Markowitz *et al.*, 2006). Groups of orthologous genes were determined using the OrthoMCL 1.4 software (<http://www.orthomcl.org/>) using the default parameters (Li *et al.*, 2003a). To determine the species phylogenetic tree, we first selected about 20 proteins from groups of orthologues that have only one member in each genome and aligned the amino acid sequences with the MUSCLE 3.7 software (<http://www.drive5.com/muscle/>) (Edgar, 2004). The alignments were then concatenated and poorly aligned positions were removed using the GBlocks 0.91b software (<http://molevol.cmima.csic.es/castresana/Gblocks.html>) (Talavera and Castresana, 2007). Finally, the phylogenetic tree was computed from the multiple alignment using the Fasttree 1.0.0 software (<http://www.microbesonline.org/fasttree/>) set to default parameters (Price *et al.*, 2009).

Comparative genomics reconstruction of the SigR regulon

The phylogenetic analyses used to predict the conservation of SigR target genes across related species was conducted as previously described (Dufour *et al.*, 2010a). The SigR promoter sequence model used to predict target genes across all the genomes sequences was constructed by aligning 58 SigR promoter sequences from *S. coelicolor* using the HMMER 1.8.5 software (<http://hmmer.janelia.org/>). To detect putative SigR-dependent promoters we scored the 300 base pair sequence upstream of every annotated gene in each genome. The distribution of scores of each genome was normalized to a normal distribution to eliminate the influence of varying base composition in the background sequences among species. In addition, because bacterial genomes are organized in polycistronic operons, the promoter scores were propagated to the remaining genes of the operon according to the probability that consecutive genes are co-transcribed. Operon predictions were obtained from the VIMSS database (<http://www.microbesonline.org/operons/>) (Price *et al.*, 2005). Finally, genes with a z-score of ≥ 2.0 were selected as putative SigR targets.

Acknowledgements

This work was supported by grants for NRL of Molecular Microbiology to J.H. Roe (NRF-2009-0079278) and for the Intelligent Synthetic Biology Center of Global Frontier Project from MEST (2011-0031960). Ji-Sun Yoo received doctoral fellowship from the second-stage BK21 Program for Life Sciences at SNU.

References

- Antelmann H, Helmann JD. Thiol-based redox switches and gene regulation. *Antioxid Redox Signal*. 2011; 14:1049–1063. [PubMed: 20626317]
- Antelmann H, Hecker M, Zuber P. Proteomic signatures uncover thiol-specific electrophile resistance mechanisms in *Bacillus subtilis*. *Expert Rev Proteomics*. 2008; 5:77–90. [PubMed: 18282125]
- Bae JB, Park JH, Hahn MY, Kim MS, Roe JH. Redox-dependent changes in RsrA, an anti-sigma factor in *Streptomyces coelicolor*: zinc release and disulfide bond formation. *J Mol Biol*. 2004; 335:425–435. [PubMed: 14672653]

- Barends S, Zehl M, Bialek S, de Waal E, Traag BA, Willemse J, et al. Transfer-messenger RNA controls the translation of cell-cycle and stress proteins in *Streptomyces*. *EMBO Rep*. 2010; 11:119–125. [PubMed: 20019758]
- Baylis HA, Bibb MJ. Transcriptional analysis of the 16S rRNA gene of the *rrnD* gene set of *Streptomyces coelicolor* A3(2). *Mol Microbiol*. 1988; 2:569–579. [PubMed: 2460716]
- Bellier A, Mazodier P. ClgR, a novel regulator of *clp* and *lon* expression in *Streptomyces*. *J Bacteriol*. 2004; 186:3238–3248. [PubMed: 15126487]
- Bieda M, Xu X, Singer MA, Green R, Farnham PJ. Unbiased location analysis of E2F1-binding sites suggests a widespread role for E2F1 in the human genome. *Genome Res*. 2006; 16:595–605. [PubMed: 16606705]
- Brandes N, Schmitt S, Jakob U. Thiol-based redox switches in eukaryotic proteins. *Antioxid Redox Signal*. 2009; 11:997–1014. [PubMed: 18999917]
- Burns KE, Darwin KH. Pupylation versus ubiquitylation: tagging for proteasome-dependent degradation. *Cell Microbiol*. 2010; 12:424–431. [PubMed: 20109157]
- Buttner MJ, Chater KF, Bibb MJ. Cloning, disruption, and transcriptional analysis of three RNA polymerase sigma factor genes of *Streptomyces coelicolor* A3(2). *J Bacteriol*. 1990; 172:3367–3378. [PubMed: 2160942]
- Christensen-Dalsgaard M, Jorgensen MG, Gerdes K. Three new RelE-homologous mRNA interferases of *Escherichia coli* differentially induced by environmental stresses. *Mol Microbiol*. 2010; 75:333–348. [PubMed: 19943910]
- D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol*. 2007; 8:813–824. [PubMed: 17848967]
- Darwin KH. Prokaryotic ubiquitin-like protein (Pup), proteasomes and pathogenesis. *Nat Rev Microbiol*. 2009; 7:485–491. [PubMed: 19483713]
- Dey A, Verma AK, Chatterji D. Role of an RNA polymerase interacting protein, MsRbpA, from *Mycobacterium smegmatis* in phenotypic tolerance to rifampicin. *Microbiology*. 2010; 156:873–883. [PubMed: 19926651]
- Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, et al. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci USA*. 2002; 99:11908–11913. [PubMed: 12193649]
- Dufour YS, Landick R, Donohue TJ. Organization and evolution of the biological response to singlet oxygen stress. *J Mol Biol*. 2008; 383:713–730. [PubMed: 18723027]
- Dufour YS, Kiley PJ, Donohue TJ. Reconstruction of the core and extended regulons of global transcription factors. *PLoS Genet*. 2010a; 6:e1001027. [PubMed: 20661434]
- Dufour YS, Wesenberg GE, Tritt AJ, Glasner JD, Perna NT, Mitchell JC, Donohue TJ. chipD: a web tool to design oligonucleotide probes for high-density tiling arrays. *Nucleic Acids Res*. 2010b; 38:W321–W325. [PubMed: 20529880]
- Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004; 32:1792–1797. [PubMed: 15034147]
- Engels S, Schweitzer JE, Ludwig C, Bott M, Schaffer S. *clpC* and *clpPIP2* gene expression in *Corynebacterium glutamicum* is controlled by a regulatory network involving the transcriptional regulators ClgR and HspR as well as the ECF sigma factor sigmaH. *Mol Microbiol*. 2004; 52:285–302. [PubMed: 15049827]
- Gust, B.; Kieser, T.; Chater, K. Redirect Technology: PCR-targeting System in *Streptomyces coelicolor*. Norwich: John Innes Centre; 2002.
- Hahn MY, Roe JH. Partial purification of factors for differential transcription of the *rrnD* promoters for ribosomal RNA synthesis in *Streptomyces coelicolor*. *J Microbiol*. 2007; 45:534–540. [PubMed: 18176537]
- Hayashi A, Suzuki H, Itoh K, Yamamoto M, Sugiyama Y. Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. *Biochem Biophys Res Commun*. 2003; 310:824–829. [PubMed: 14550278]
- den Hengst CD, Buttner MJ. Redox control in actinobacteria. *Biochim Biophys Acta*. 2008; 1780:1201–1216. [PubMed: 18252205]

- Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol.* 2005; 6:150–166. [PubMed: 15688001]
- Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics.* 2002; 18(Suppl. 1):S96–S104. [PubMed: 12169536]
- Imlay JA. Cellular defenses against superoxide and hydrogen peroxide. *Annu Rev Biochem.* 2008; 77:755–776. [PubMed: 18173371]
- Jacob C, Knight I, Winyard PG. Aspects of the biological redox chemistry of cysteine: from simple redox responses to sophisticated signalling pathways. *Biol Chem.* 2006; 387:1385–1397. [PubMed: 17081111]
- Kallifidas D, Thomas D, Doughty P, Paget MS. The sigmaR regulon of *Streptomyces coelicolor* A32 reveals a key role in protein quality control during disulphide stress. *Microbiology.* 2010; 156:1661–1672. [PubMed: 20185507]
- Kang JG, Paget MS, Seok YJ, Hahn MY, Bae JB, Hahn JS, et al. RsrA, an anti-sigma factor regulated by redox change. *EMBO J.* 1999; 18:4292–4298. [PubMed: 10428967]
- Kawanishi S, Hiraku Y, Oikawa S. Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat Res.* 2001; 488:65–76. [PubMed: 11223405]
- Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol.* 2007; 47:89–116. [PubMed: 16968214]
- Kieser, T.; Bibb, M.; Buttner, M.; Chater, K.; Hopwood, D. *Practical Streptomyces Genetics*. Norwich: The John Innes Foundation; 2000.
- Kiley PJ, Storz G. Exploiting thiol modifications. *PLoS Biol.* 2004; 2:e400. [PubMed: 15547642]
- Kim MS, Hahn MY, Cho Y, Cho SN, Roe JH. Positive and negative feedback regulatory loops of thiol-oxidative stress response mediated by an unstable isoform of sigmaR in actinomycetes. *Mol Microbiol.* 2009; 73:815–825. [PubMed: 19682253]
- Kwak MK, Wakabayashi N, Greenlaw JL, Yamamoto M, Kensler TW. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol Cell Biol.* 2003; 23:8786–8794. [PubMed: 14612418]
- Li L, Stoeckert CJ, Roos DS. OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res.* 2003a; 13:2178–2189. [PubMed: 12952885]
- Li W, Bottrill AR, Bibb MJ, Buttner MJ, Paget MS, Kleanthous C. The role of zinc in the disulphide stress-regulated anti-sigma factor RsrA from *Streptomyces coelicolor*. *J Mol Biol.* 2003b; 333:461–472. [PubMed: 14529630]
- Manganelli R, Voskuil MI, Schoolnik GK, Dubnau E, Gomez M, Smith I. Role of the extracytoplasmic-function sigma factor sigma(H) in *Mycobacterium tuberculosis* global gene expression. *Mol Microbiol.* 2002; 45:365–374. [PubMed: 12123450]
- Markowitz VM, Korzeniewski F, Palaniappan K, Szeto E, Werner G, Padki A, et al. The integrated microbial genomes (IMG) system. *Nucleic Acids Res.* 2006; 34:D344–D348. [PubMed: 16381883]
- Martin B, Garcia P, Castanie MP, Claverys JP. The *recA* gene of *Streptococcus pneumoniae* is part of a competence-induced operon and controls lysogenic induction. *Mol Microbiol.* 1995; 15:367–379. [PubMed: 7538190]
- Neubauer C, Gao YG, Andersen KR, Dunham CM, Kelley AC, Hentschel J, et al. The structural basis for mRNA recognition and cleavage by the ribosome-dependent endonuclease RelE. *Cell.* 2009; 139:1084–1095. [PubMed: 20005802]
- Newell KV, Thomas DP, Brekasis D, Paget MS. The RNA polymerase-binding protein RbpA confers basal levels of rifampicin resistance on *Streptomyces coelicolor*. *Mol Microbiol.* 2006; 60:687–696. [PubMed: 16629670]
- Newton GL, Buchmeier N, Fahey RC. Biosynthesis and functions of mycothiol, the unique protective thiol of *Actinobacteria*. *Microbiol Mol Biol Rev.* 2008; 72:471–494. [PubMed: 18772286]
- Nguyen TT, Eiamphungporn W, Mader U, Liebeke M, Lalk M, Hecker M, et al. Genome-wide responses to carbonyl electrophiles in *Bacillus subtilis*: control of the thiol-dependent formaldehyde dehydrogenase AdhA and cysteine proteinase YraA by the MerR-family regulator YraB (AdhR). *Mol Microbiol.* 2009; 71:876–894. [PubMed: 19170879]

- Owen GA, Pascoe B, Kallifidas D, Paget MS. Zinc-responsive regulation of alternative ribosomal protein genes in *Streptomyces coelicolor* involves zur and sigmaR. *J Bacteriol.* 2007; 189:4078–4086. [PubMed: 17400736]
- Paget MS, Buttner MJ. Thiol-based regulatory switches. *Annu Rev Genet.* 2003; 37:91–121. [PubMed: 14616057]
- Paget MS, Kang JG, Roe JH, Buttner MJ. sigmaR, an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). *EMBO J.* 1998; 17:5776–5782. [PubMed: 9755177]
- Paget MS, Molle V, Cohen G, Aharonowitz Y, Buttner MJ. Defining the disulphide stress response in *Streptomyces coelicolor* A3(2): identification of the sigmaR regulon. *Mol Microbiol.* 2001; 42:1007–1020. [PubMed: 11737643]
- Park JH, Roe JH. Mycothiol regulates and is regulated by a thiol-specific antisigma factor RsrA and sigma(R) in *Streptomyces coelicolor*. *Mol Microbiol.* 2008; 68:861–870. [PubMed: 18430082]
- Pearce MJ, Mintseris J, Ferreyra J, Gygi S, Darwin KH. Ubiquitin-like protein involved in the proteasome pathway of *Mycobacterium tuberculosis*. *Science.* 2008; 322:1104–1107. [PubMed: 18832610]
- Perez JC, Groisman EA. Evolution of transcriptional regulatory circuits in bacteria. *Cell.* 2009; 138:233–244. [PubMed: 19632175]
- Price MN, Huang KH, Alm EJ, Arkin A. A novel method for accurate operon predictions in all sequenced prokaryotes. *Nucleic Acids Res.* 2005; 33:880–892. [PubMed: 15701760]
- Price MN, Dehal PS, Arkin A. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol Biol Evol.* 2009; 26:1641–1650. [PubMed: 19377059]
- Raman S, Song T, Puyang X, Bardarov S, Jacobs WR, Husson RN. The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in *Mycobacterium tuberculosis*. *J Bacteriol.* 2001; 183:6119–6125. [PubMed: 11567012]
- Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, et al. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J Clin Invest.* 2004; 114:1248–1259. [PubMed: 15520857]
- Rudolph TK, Freeman BA. Transduction of redox signaling by electrophile-protein reactions. *Sci Signal.* 2009; 2:re7. [PubMed: 19797270]
- Sachdeva P, Misra R, Tyagi AK, Singh Y. The sigma factors of *Mycobacterium tuberculosis*: regulation of the regulators. *FEBS J.* 2010; 277:605–626. [PubMed: 19951358]
- Shin JH, Oh SY, Kim SJ, Roe JH. The zinc-responsive regulator Zur controls a zinc uptake system and some ribosomal proteins in *Streptomyces coelicolor* A3(2). *J Bacteriol.* 2007; 189:4070–4077. [PubMed: 17416659]
- Sinha RP, Hader D. UV-induced DNA damage and repair: a review. *Photochem Photobiol Sci.* 2002; 1:225–236. [PubMed: 12661961]
- Song T, Song SE, Raman S, Anaya M, Husson RN. Critical role of a single position in the –35 element for promoter recognition by *Mycobacterium tuberculosis* SigE and SigH. *J Bacteriol.* 2008; 190:2227–2230. [PubMed: 18192397]
- Synold T, Xi B, Wuenschell GE, Tamae D, Figarola JL, Rahbar S, Termini J. Advanced glycation end products of DNA: quantification of N2-(1-Carboxyethyl)-2'-deoxyguanosine in biological samples by liquid chromatography electrospray ionization tandem mass spectrometry. *Chem Res Toxicol.* 2008; 21:2148–2155. [PubMed: 18808156]
- Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol.* 2007; 56:564–577. [PubMed: 17654362]
- Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res.* 2002; 62:5196–5203. [PubMed: 12234984]
- Yang YH, Song E, Kim EJ, Lee K, Kim WS, Park SS, et al. NdgR, an IclR-like regulator involved in amino-acid-dependent growth, quorum sensing, and antibiotic production in *Streptomyces coelicolor*. *Appl Microbiol Biotechnol.* 2009; 82:501–511. [PubMed: 19083232]

Zuber P. Management of oxidative stress in *Bacillus*. *Annu Rev Microbiol.* 2009; 63:575–597.
[PubMed: 19575568]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

S1 nuclease mapping of selected SigR-dependent promoter regions. RNA samples were prepared from exponentially growing wild-type and *sigRrsrA* cells treated with 0.5 mM diamide for 0, 20, 40 and 80 min.

A. Transcript patterns of genes whose primary transcripts are from SigR-dependent promoters.

B. Results from genes which produce transcripts from other promoters independent of SigR. The bands with marked asterisks in panel B are from predicted SigR-binding promoters.

Fig. 2.
SigR-dependent transcription activation of the *hrdB* gene encoding the major sigma factor in *S. coelicolor*.
A. The position of the SigR-dependent promoter p2 upstream of the *hrdB* coding region predicted from ChIP-chip and sequence pattern. The previously reported promoter p1 resides further upstream.
B. S1 nuclease mapping of *hrdB* transcripts from diamide-treated cells revealing two alternative transcripts.
C. S1 nuclease mapping of *hrdB* transcripts from diamide-treated cells revealing two alternative transcripts.

C. Western blot analysis of HrdB protein. Crude extracts from cells treated with diamide (0.1 mM) for up to 120 min were analysed to quantify the amount of HrdB protein as described in *Experimental procedures*. For each lane, an aliquot of 1 µg protein extract plus 1 µg BSA in lysis buffer was analysed. Results from four independent experiments were quantified to estimate changes in the level of HrdB protein upon oxidative stress, presented with the average value $\pm$ standard deviations (SD).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 3.

Sensitivity of *uvrA* and *sigRrsrA* mutants to UV and thiol-reactive oxidants and electrophiles. Serially diluted spores of wild-type (WT), *sigRrsrA*, *uvrA* strains were spotted on NA plates with or without added chemicals such as diamide (0.6 mM), methyl hydroquinone (MHQ, 1 mM) and methyl glyoxal (MG, 1 mM). For UV irradiation, the spotted plates were exposed to UV light from UV cross-linker for 30 seconds. Plates were incubated at 30°C for 1 (untreated), 2 (UV, diamide), 5 (MHQ) or 6 days (MG).

Mol Microbiol. Author manuscript; available in PMC 2015 August 22.

Orange and yellow indicate moderate and strong match to the SigR promoter model respectively. Black indicates that the corresponding species possess a gene belonging to the corresponding orthologous set but that no match to the SigR promoter model, while grey indicates that the species lacks a gene representing the orthologous set. Species are organized according to their phylogenetic relationship as indicated by the dendrogram on the right. Sets of orthologous genes are identified by arbitrary numbers (Table 2). Sets of orthologous genes containing confirmed SigR target genes in *S. coelicolor* are indicated by circles at the bottom. Red circles indicate genes detected by SigR ChIP-chip and experimentally verified for SigR-dependent expression. The black circle indicates a gene that is associated with a SigR ChIP-chip peak without experimental verification.

Fig. 5.

The top 250 orthologous sets of genes predicted to be SigR targets across 42 selected *Actinomycetes*. The heatmap colour coding is identical to Fig. 4. Species are organized in the same order as in Fig. 4. Red marks indicate genes that are associated with a SigR ChIP-chip peak and differentially expressed under diamide stress, while black marks indicate genes that are only associated with a SigR ChIP-chip peak. The broader characterization of the conservation of SigR targets predicts in addition to the core regulon the existence of extended SigR regulons that are specific to lineages of related species. Lineage specific SigR regulons are indicated by letters on top, and the gene composition of each group is summarized in Table S2.

Fig. 6. Induction and function of SigR regulon in comparison with mammalian anti-oxidative Nrf2-Keap1 system. SigR regulon is induced in response to thiol-reactive oxidants and electrophilic compounds, through inactivation of antisigma RsrA by forming intramolecular disulphide bond. Nrf2 regulon is induced by similar stressors, through inactivation of inhibitory factor Keap1 that facilitates degradation of Nrf2, by forming multiple intra- and inter-molecular disulphide bonds, and S-alkylation. Functions of SigR target genes overlap with those of Nrf2 targets to a large extent, which include thiol redox homeostasis, small thiol-mediated detoxification, protein quality control, and pentose phosphate pathway for generating NADPH. The scheme for Nrf2 activation mechanism is simplified to highlight analogy with SigR-RsrA (Kensler *et al.*, 2007; Antelmann and Helmann, 2011).

Table 1

The SigR regulon genes.

<i>G^a</i>	<i>Number^b</i>	<i>Name</i>	<i>Description</i>	<i>Confirm^c</i>
3	SCO0569	<i>rpmJ</i>	50S ribosomal protein L36	
3	SCO0570*	<i>rpmG3</i>	50S ribosomal protein L33 (C-type)	(S1)
8	SCO0882*		Hypothetical protein, possible dithiol-disulphide isomerase	(S1)
8	SCO0884*		Probable oxidoreductase, probable pyridine nucleotide-disulphide oxidoreductases	
1	SCO0885*	<i>trxC</i>	Thioredoxin	(S1, A)
8	SCO0917*		Possible oxygenase	
7	SCO0973*		Probable integral membrane protein	
1	SCO1084*	<i>trxA4</i>	Putative thioredoxin	S1
12	SCO1085*		Probable phospholipid/glycerol acyltransferase, divergent from trxA4 (1084)	
8	SCO1142*		Possible oxidoreductase, probable FAD-dependent pyridine nucleotide-disulphide oxidoreductase	
5	SCO1238*		Putative ATP-dependent Clp protease subunit	
6	SCO1425*		Possible AsnC-family transcriptional regulatory protein	
14	SCO1426*		Hypothetical protein	
3	SCO1513*	<i>relA</i>	GTP pyrophosphokinase for ppGpp synthesis	(S1)
3	SCO1598	<i>rplT</i>	50S ribosomal protein L20	
3	SCO1599	<i>rpmI</i>	50S ribosomal protein L35	
3	SCO1600*	<i>infC</i>	Translation initiation factor IF-3	S1
6	SCO1618		Hypothetical protein, similar to cobalamin binding protein BtuF	
6	SCO1619*		Possible transcriptional regulatory protein of HTH-XRE family	
5	SCO1643	<i>prcA</i>	20S proteasome alpha-subunit	
5	SCO1644	<i>prcB</i>	20S proteasome beta-subunit	
5	SCO1645		Hypothetical protein	
5	SCO1646	<i>pup</i>	Prokaryotic ubiquitin-like protein	
5	SCO1647	<i>pafD</i>	Proteasome accessory factor	
5	SCO1648*	<i>mpa</i>	Probable AAA ATPase similar to mycobacterium proteasome-associated ATPase	S1
3	SCO1758*	<i>engA</i>	GTP-binding protein with multiple functions	S1
8	SCO1869*		Conserved hypothetical, probable protein dithiol-disulphide isomerase	(S1, A)
11	SCO1919		Probable metal-sulphur cluster biosynthetic enzyme	(A)
11	SCO1920*	<i>sufU</i>	Similar to NifU in SUF-type Fe-S assembly gene cluster (1926-1919)	(A), S1
9	SCO1936*	<i>tal2</i>	Probable transaldolase, a component of non-oxidative pentose phosphate pathway	S1
9	SCO1937	<i>zwf2</i>	Glucose 6-phosphate 1-dehydrogenase	
9	SCO1938		Hypothetical protein	
9	SCO1939	<i>pgl</i>	6-phosphogluconolactonase	
10	SCO1958*	<i>uvrA</i>	ABC excision nuclease subunit A	(A), S1
14	SCO1995*		Unknown	(S1, A)

G^a	Number^b	Name	Description	Confirm^c
11	SCO1996	<i>coaE</i>	Dephospho-CoA kinase	(A)
11	SCO1997*		Unknown	(S1, A)
7	SCO2124*		Possible hydrophobic membrane spanning regions	
7	SCO2154*		Integral membrane protein	
11	SCO2161*	<i>sufA</i>	HesB/YadR/Yfh family protein, probable SufA	(S1), S1
11	SCO2162*	<i>nadA</i>	Quinolinate synthetase A subunit for nicotinamide synthesis, divergent from sufA (2161). 4Fe-4S enzyme	
11	SCO2194*	<i>lipA</i>	Putative lipoyl synthase	S1
7	SCO2254*		Possible transmembrane efflux protein of MFS(major facilitator superfamily)	S1
7	SCO2310*		Possible transmembrane efflux protein similar to TcmA	
6	SCO2331*		Possible MarR family transcriptional regulator	
6	SCO2481*		Hypothetical protein, putative transcriptional regulator	
6	SCO2537*		Possible DNA-binding protein	(A), S1
6	SCO2538		Hypothetical protein	
6	SCO2539	<i>era</i>	GTP-binding protein era	
3	SCO2595*	<i>obgE</i>	GTPase, Obg subfamily, ObgE	S1
5	SCO2617	<i>clpX</i>	ATP-dependent Clp protease ATP binding subunit	
5	SCO2618	<i>clpP2</i>	ATP-dependent Clp protease proteolytic subunit 2	(A)
5	SCO2619*	<i>clpP1</i>	ATP dependent Clp protease proteolytic subunit 1	(S1, A)
8	SCO2634*		Hypothetical protein, contains DsbA-like thioredoxin domain	(S1, A)
5	SCO2635*		Probable aminopeptidase	
14	SCO2642*		Unknown	
5	SCO2643*	<i>pepN</i>	Aminopeptidase N	(S1, A)
7	SCO2763*		ABC transporter protein, ATP-binding component	S1
8	SCO2816*		Conserved hypothetical protein, flavin utilizing monooxygenase superfamily	
8	SCO2849*		Conserved hypothetical protein, flavin utilizing monooxygenase superfamily	(S1, A)
2	SCO2910	<i>cysM</i>	Cysteine synthase	(A)
2	SCO2911*	<i>moaD</i>	Putative MoaD-family protein with ubiquitin fold	(A)
3	SCO302*	<i>ssrA</i>	Transfer messenger RNA (tmRNA)	(S1)
7	SCO3083*		Possible integral membrane protein	(S1, A)
12	SCO3091*	<i>cfa</i>	Cyclopropane-fatty-acyl-phospholipid synthase, SAM-dependent methylase	(A), S1
13	SCO3162*		Possible esterase, β -lactamase superfamily	(S1, A)
14	SCO3187*		Hypothetical protein	(S1)
7	SCO3206*		Probable transmembrane efflux protein	(A)
6	SCO3207*		TetR family transcriptional regulator	
8	SCO3295		Possible oxidoreductase	
8	SCO3296*		Possible oxidoreductase, similar to F420-dependent alcoholdehydrogenase	S1

G^a	Number^b	Name	Description	Confirm^c
5	SCO3373*	<i>clpC</i>	Probable Clp-family ATP-binding protease similar to MecB/ClpC	(S1), S1
11	SCO3403*	<i>folE</i>	GTP cyclohydrolase I for tetrahydrofolate biosynthesis	(S1)
1	SCO3442*	<i>mrxB</i>	Glutaredoxin-like protein, probable mycoredoxin	S1
6	SCO3449	<i>rsrA2-1</i>	Putative ZAS (zinc-containing anti-sigma) factor	
6	SCO3450*	<i>sigR2</i>	ECF (group 4) sigma factor close to SigR	
6	SCO3451*	<i>rsrA2-1</i>	Putative ZAS (zinc-containing anti-sigma) factor	
14	SCO3509*		Hypothetical protein	
7	SCO3764		Integral membrane protein	(A)
7	SCO3765*		Possible integral membrane protein with CBS domain	
14	SCO3766*		Hypothetical protein	
14	SCO3767*		Hypothetical protein, similar to tellurite resistance protein TerB	
1	SCO3889	<i>trxA</i>	Thioredoxin	(A)
1	SCO3890*	<i>trxB</i>	Thioredoxin reductase	(S1, A)
14	SCO4039*		Unknown	(A)
14	SCO4040*		Unknown	
8	SCO4109*		Possible oxidoreductase, probable aldo/keto reductases	
14	SCO4203*		Hypothetical protein	
1	SCO4204*	<i>mshA</i>	Glycosyltransferase for mycothiol synthesis	(S1, A)
1	SCO4205		Conserved hypothetical protein	
8	SCO4297*		Possible oxidoreductase with FMN binding site, old yellow enzyme family	(A)
8	SCO4298		Carboxylesterase	
8	SCO4299		Integral membrane protein	
11	SCO4418	<i>pdxH2</i>	Pyridoxamine 5'-phosphate oxidase oxidase	
11	SCO4419*	<i>pdxH3</i>	Conserved hypothetical, probable pyridoxamine 5'-phosphate oxidase for pyridoxal phosphate synthesis	
14	SCO4420*		Hypothetical protein	
13	SCO4561*		NLP/P60 family protein	
4	SCO4770*	<i>guaB</i>	Inosine 5' monophosphate dehydrogenase	(S1, A)
4	SCO4771	<i>guaB1</i>	Inosine 5' monophosphate dehydrogenase	
10	SCO4797*	<i>uvrD</i>	Probable ATP-dependent DNA helicase	S1
8	SCO4833		Phosphorylmutase	
8	SCO4834		Hypothetical protein	
8	SCO4835*		Conserved hypothetical protein with alkylhydroperoxidase-like AhpD/CMD domain	
2	SCO4956*	<i>msrA</i>	Probable peptide methionine S-sulphoxide reductase	(S1, A), S1
14	SCO4966*		Possible membrane protein	
1	SCO4967*	<i>mca</i>	Mycothiol-S-conjugate amidase	(S1, A)
1	SCO4968		Hypothetical protein	(A)
9	SCO5042*	<i>fumC</i>	Fumarate hydratase, class II family (iron-independent)	(A)

G^a	Number^b	Name	Description	Confirm^c
	6	SCO5065*	Possible transcriptional regulator, LuxR family	
	14	SCO5163*	Unknown	(S1)
	11	SCO5178* <i>moeB</i>	Possible sulphurylase related to molybdopterin synthesis	(S1, A)
	1	SCO5187* <i>mrxA</i>	Glutaredoxin-like protein, probable myc917oredoxin	(S1, A)
	10	SCO5188* <i>uvrD2</i>	ATP-dependent DNA helicase (UvrD/REP type), divergent from <i>mrxA</i> (5187)	
	6	SCO5216* <i>sigR</i>	ECF (group 4) sigma factor s ^R	(S1, A)
	6	SCO5217 <i>rsrA</i>	ZAS (zinc-containing antisigma) factor for s ^R	(A)
	14	SCO5284*	Hypothetical protein	
	5	SCO5285* <i>lon</i>	ATP-dependent protease	(S1)
	6	SCO5357* <i>rho</i>	Transcription termination factor, an RNA–DNA helicase	
	3	SCO5359* <i>rpmE1</i>	Ribosomal proteins L31	(S1, A)
	3	SCO5360 <i>prfA</i>	Peptide release factor A	
	3	SCO5361 <i>hemK</i>	Probable release factor-specific methyltransferase	
	8	SCO5465*	Conserved hypothetical, probable F420-dependent NADP oxidoreductase	(S1, A)
	14	SCO5490*	Hypothetical protein	
	14	SCO5545*	hypothetical protein	(A)
	6	SCO5552* <i>ndgR</i>	IcIR family transcription regulator	
	3	SCO5705*	Hypothetical protein, first gene in translation-related gene cluster	S1
	3	SCO5706 <i>infB</i>	Translation initiation factor IF-2	
	3	SCO5707	Hypothetical protein	
	3	SCO5708 <i>rbfA</i>	Ribosome binding factor	
	3	SCO5709 <i>truB</i>	Probable tRNA pseudouridine synthase	
	10	SCO5754* <i>cinA</i>	Competence-DNA damage-induced protein, neighbouring <i>clgR</i> (5755)	(S1, A)
	3	SCO5796* <i>hflX</i>	Conserved ribosome binding GTPase	(S1, A)
	6	SCO5820* <i>hrdB</i>	Major vegetative sigma factor	S1
	14	SCO5864*	Hypothetical protein	
	14	SCO5865	Hypothetical protein	
	2	SCO6061* <i>msrB</i>	Probable peptide methionine R-sulphoxide reductase	(S1, A), S1
	14	SCO6126*	Hypothetical protein	
	13	SCO6127*	Probable carboxylesterase, probable phenylcarbamate hydrolase	
	11	SCO6423*	Putative lipoate-protein ligase	(A)
	8	SCO6551*	Probable oxidoreductase, aldo/keto reductase family	(S1, A)
	12	SCO6759* <i>phyA/hopE</i>	Probable phytoene (hopanoid) synthase	(S1, A), S1
	12	SCO6760 <i>phyB/hopD</i>	Squalene/phytoene synthase	
	12	SCO6761	Small (52 aa) hypothetical protein of unknown function	
	12	SCO6762 <i>phyC/hopC</i>	Squalene/phytoene dehydrogenase	
	12	SCO6763 <i>phyD/hopB</i>	Polyprenyl diphosphate synthase	

G^a	Number^b	Name	Description	Confirm^c
12	SCO6764	<i>phyE/hopA</i>	Squalene–hopene cyclase	
12	SCO6765		Lipoprotein	
12	SCO6766		Hypothetical protein	
12	SCO6767	<i>ispG</i>	4-hydroxy-3-methyl-2-en-1-yl diphosphate synthase	
12	SCO6768		1-deoxy-D-xylulose-5-phosphate synthase	
12	SCO6769		Aminotransferase	
12	SCO6770		DNA-binding protein	
12	SCO6771		Small hydrophobic protein	
6	SCO6775*		Probable transcriptional regulator, TetR family	
14	SCO6776*		Unknown	
6	SCO7140*		Probable DNA-binding protein	
14	SCO7631*		Possible secreted protein	
1	SCO7632*		Probable amidase similar to <i>mshB</i> and <i>mca</i>	(A), S1
8	SCO7784*		Possible oxidoreductase, probable nitro/flavin reductase	S1
8	SCO7785		Transcriptional regulator	

^a Genes were grouped according to predicted functions such as thiol homeostasis (1), sulphur metabolism (2), modulation of ribosome and/or translation (3), guanine nucleotide metabolism (4), protein degradation (5), transcriptional regulators or DNA binding proteins (6), transporter or integral membrane proteins (7), oxidoreductases (8), energy metabolism (9), DNA damage repair and/or recombination (10), cofactor metabolism (11), lipid metabolism (12), others (13), or function unpredictable (14).

^b Genes with SigR-binding promoters were marked with asterisks (*).

^c The genes whose SigR-dependent expression was previously confirmed by S1 or microarray (A) were marked with S1 and A in parenthesis (Paget *et al.*, 2001; Park and Roe, 2008; Kallifidas *et al.*, 2010). Those confirmed in this study were noted with S1.

Table 2

Top 25 genes that are highly conserved as SigR regulon members among actinomycetes.

Orthologues No.	Gene No. ^a	Gene name	Function	Genus (frequency) ^b
61	SCO3671	<i>dnaK</i>	Hsp70 family chaperone, no direct SigR target in Sco, First gene in dnaK-grpE-dnaJ-hspR cluster (3671-3668), a SigE target in Mtb	Co, My, No, Rh, Sc, F, T, N, Ki, J, Mi, B, Ac, P (14/22)
445	SCO3670	<i>grpE</i>	Co-chaperone	Co, My, No, Rh, Sc, F, T, N, Ki, J., B, Ac (12/22)
852	SCO3083*		Possible integral membrane protein	Co, My, No, Rh, Sc, F, St, T, N, Ki, B, P (12/22)
236	SCO5360*	<i>prfA</i>	Peptide chain release factor 1 (RF-1), rpmE1-prfA operon	My, Rh, Sc, F, St, T, N, Ki (8/22)
2534	MT2783	<i>Mtb-sigB</i>	Sigma factor SigB in <i>M. tuberculosis</i> regulated by SigE	Co, My, No, Rh, Sc (5/22)
1014	SCO2634*		Hypothetical protein, contains DsbA-like thioredoxin domain	Co, My, No, Rh, Sc, F, St, T, N, Ar, Re (11/22)
1035	SCO3187*		Hypothetical protein	My, No, Rh, F, St, J, Ar, Re (8/22)
646	SCO5755*	<i>clgR</i>	Activator of clpP and lon protease synthetic genes. Neighbouring a SigR-target gene (5754; cinA)	Co, My, No, Rh, Sc, St, N, Ki, Ar, Ko, Mi, Cl, Ag, Ac, P (15/22)
217	SCO0885*	<i>trxC</i>	Thioredoxin	Co, My, No, Rh, Sa, St, Ac, N, Ki, Ko, Mi, Ag, Ac, P (14/22)
882	SCO1997*		Unknown	Co, My, No, Rh, Sa, F, St, J, Ar, Re, Ko, Mi, P (13/22)
677	SCO2161*	<i>sufA</i>	HesB domain, probable Iron-sulphur cluster assembly protein	Co, My, No, Rh, Sc, Sa, St, T, N, Ki, J, Ar, Re, Ko, Mi, Cl, B (17/22)
598	SCO1421	<i>rpba</i>	RNA polymerase binding protein	Co, My, No, Rh, Sc, Sa, F, St, Ac, T, N, Ki, J, Ar, Re, Ko, Mi, B (18/22)
838	SCO2816 [#]		Conserved hypothetical protein, Flavin utilizing monooxygenase superfamily	Co, My, No, Rh, Sc, F, St, Ki, J, Ar, Ko, Mi, Cl, B (14/22)
80	SCO3890*	<i>trxB</i>	Thioredoxin reductase	Co, My, No, Rh, Sc, Sa, F, St, Ac, T, Ki, J, Ar, Re, Ko, Mi, L, Cl, B, Ac, P (21/22)
643	SCO5216*	<i>sigR</i>	Sigma factor R	Co, My, No, Rh, Sc, Sa, F, St, Ac, T, N, Ki, J, Ar, Re, Ko, Mi, Cl, B, Ac, P (21/22)
1077	SCO5217*	<i>rsrA</i>	Anti-SigR factor	Co, My, No, Rh, Sc, Sa, F, St, Ac, T, N, Ki, J, Ar, Re, Ko, Mi, Cl, Bl, P (20/22)
1	SCO3373*	<i>clpC</i>	Clp-family ATP-binding protease similar to MecB/ClpC	Co, My, No, Rh, Sc, Sa, F, St, Ac, T, N, Ki, J, Mi, L, Cl, B, Ac (18/22)
37	SCO5359*	<i>rpmE1</i>	Ribosomal proteins L31	My, Rh, Sc, Sa, F, St, T, N, Ki, J (10/22)
723	SCO4609	<i>htpX</i>	Possible peptidase, <i>M. tuberculosis</i> HtpX homologue, no SigR target, a SigE target in Mtb	My, No, Rh, Sc, F, St, N, Ki, J, Ar (10/22)
927	SCO5187*	<i>mrxA</i>	Mycoredoxin, glutaredoxin-like protein	My, No, Rh, Sa, F, St, Ac, N, Ki, Ar, Re (11/22)
406	SCO5796*		Unknown	Co, My, Sa, F, St, T, Ki, L, Cl, Ag (10/22)
157	SCO4956*	<i>msrA</i>	Methionine S-sulphoxide reductase	Co, No, F, St, N, Ki, J, Ar, Ko, Mi, Cl, Ag, B, P (14/22)
1779	SCO3296*		Possible oxidoreductase similar to F420-dependent alcoholdehydrogenase	No, Rh, Sc, St, T, N, Ki, J, Ar, Ko, Mi, L, B (13/22)
1973	SCO3295*		Possible oxidoreductase containing flavin	No, St, T, N, Ki, J, Ar, Re, Mi, L, B (11/22)

Orthologues No.	Gene No. ^a	Gene name	Function	Genus (frequency) ^b
1783	SCO1869*		Conserved hypothetical protein, probable DsbA-like dithiol-disulphide isomerase (FrnE subfamily)	Co, Rh, Sa, F, St, T, N, Ki, J, Ar, Re, Cl, Ag, B (14/22)

^aThe representative gene from *S. coelicolor* (SCO) or *M. tuberculosis* (MT) in each orthologue cluster was presented. The SCO genes that were determined to be SigR-dependent by ChIP-chip and supported by additional S1 and microarray data are marked with asterisks (*). The genes detected by ChIP-chip but not by additional expression data are marked with '#'.

^bThe genera that possess the corresponding SigR target gene in each orthologous cluster are presented with abbreviations; Co (*Corynebacterium*), My (*Mycobacterium*), No (*Norcadia*), Rh (*Rhodococcus*), Sc (*Saccharopolyspora*), Sa (*Salinispora*), F (*Frankia*), St (*Streptomyces*), T (*Thermobifida*), N (*Nocardioides*), Ki (*Kineococcus*), J (*Janibacter*), Ar (*Arthrobacter*), Re (*Renibacterium*), Ko (*Kocuria*), Mi (*Micrococcus*), L (*Leifsonia*), Cl (*Clavibacter*), Ag (*Agreia*), B (*Brevibacterium*), Ac (*Actinomyces*), and P (*Propionibacterium*). The frequency in parenthesis represents the number of genera that possess the corresponding SigR target gene among 22 genera, whose 42 genomes were examined in this study.