A genomic island of *Streptomyces coelicolor* with the self-contained regulon of an ECF sigma factor

Camilla M. Kao,[a,□]# Nitsara Karoonuthaisiri,[a,□1] David Weaver,[a,□2] Jonathan A. Vroom,[a,□3] Shuning A. Gai,[a,□4] Mai-Lan Ho,[a,□5] and Kedar G. Patel[a]

[a]Department of Chemical Engineering, Stanford University, Stanford, California, USA

Running Head: GI 6 of *S. coelicolor* with ECF sigma factor regulon

#Address correspondence to Camilla M. Kao, cammy@stanford.edu.

□ Present Address: School of Engineering, Stanford University, Stanford, California, USA

□1 Present Address: Biosensing Technology Research Unit, National Center for Engineering and Biotechnology, Pathum Thani, Thailand

□2 Present Address: ERM: Environmental Resources Management, San Francisco, California, USA

□3 Present Address: Molecular and Cellular Engineering, Codexis, Redwood City, California, USA

18 □4 Present Address: Molecular Biology, Prothena Biosciences, South San Francisco, California,
19 USA
20 □5 Present Address: Department of Radiology, Mayo Clinic, Rochester, Minnesota, USA
21 N.K. and D.W. contributed equally to this work.
22
23 Word count for the abstract: 400
24 Word count for the text: 2897
25
26 **Abstract**

27 Streptomycetes constitute the largest genus of actinobacteria, living predominantly in soil and
28 decaying vegetation. The bacteria are widely known for their filamentous morphologies and
29 their capacity to synthesize antibiotics and other biologically active molecules. More than a
30 decade ago, we and others identified 22 genomic islands that *Streptomyces coelicolor* M145
31 possesses and other *Streptomyces* strains lack. One of these genomic islands, Genomic Island
32 (GI) 6, encodes an extracytoplasmic function (ECF) sigma factor that we were characterizing in
33 separate work. Here we report that artificial induction of the ECF sigma factor, which is
34 encoded by SCO3450, causes the transcription of approximately one-fourth of GI 6, or ~26
35 mostly contiguous genes, to increase. More than half of the regulon encodes putative enzymes
36 involved in small molecule metabolism. A putative haloacid dehalogenase is present. Genes
37 encoding two putative anti-sigma factors flank SCO3450, the three genes residing within the

38 regulon. Our data suggest that the ECF sigma factor and its regulon are a self-contained
39 transcriptional unit that can be transferred by horizontal gene transfer. To our knowledge, only
40 one other example has been identified of an ECF sigma factor and its contiguous regulon
41 appearing to be transferrable by horizontal gene transfer [18,19]. Because the regulon appears
42 not to be induced by the 44 growth conditions recently examined by Byung-Kwan Cho and
43 colleagues [20], if it confers fitness to *S. coelicolor*, the regulon likely does so in as-yet unknown
44 situations. Those situations might range from scavenging to detoxification to even
45 communication within microbial communities.

46

47 **IMPORTANCE** *Streptomyces* bacteria grow as hyphae that colonize soil and differentiate into
48 spores when nutrients become scarce. In their terrestrial habitats, the bacteria encounter
49 diverse conditions. Presumably so that the bacteria can cope with those conditions, the
50 chromosomes of streptomycetes are highly dynamic, varying greatly in structure not only
51 between species but also between closely related strains of a single species. The bacteria also
52 have large numbers of extracytoplasmic function (ECF) sigma factors, which undoubtedly help
53 the microorganisms respond to the plethora of challenges coming from the environment. This
54 work illustrates these two threads of *Streptomyces* biology dovetailing: Genetic adaptability
55 through horizontal gene transfer seems to have enabled *Streptomyces coelicolor* to acquire a
56 self-contained transcriptional unit that consists of an ECF sigma factor and its regulon. The
57 suggested facile movement of the regulon between microbial hosts indicates the value of the
58 metabolism of small molecules possibly mediated by the regulon.

59

60 **Introduction**

61 Researchers began studying *Streptomyces* genetics in the second half of the 20th century
62 because of the filamentous morphologies and biosynthetic capacities of the bacteria [1]. Many
63 of the *Streptomyces* strains that were studied belong to a group of closely related "blue"
64 streptomycetes that had been given various names, but probably should all have the same
65 name [2]. During the early 2000's, we characterized the genomes of six of these "blue" strains
66 by using DNA microarrays: the sequenced strain [3], *S. coelicolor* M145, which derives from *S.*
67 *coelicolor* A3(2), which in turn derives from Waksman's strain 3443 [4]; Sermonti's SE1 (John
68 Innes (JI) strain 1152) [5]; Bradley's S199 (JI 1153) [6]; *S. lividans* 66 (JI 1326) [7]; *S. lividans*
69 ISP5434 (JI 2896), which contains the plasmid pIJ101 [8]; and *S. violaceoruber* SANK95570 (JI
70 3034), which contains the plasmid pSV1 that encodes the biosynthetic genes for the antibiotic
71 methylenomycin [9]. That study was described in the doctoral dissertation of co-author David
72 Weaver [10]. Similar findings were reported by Jayapal et al., who examined *S. coelicolor* M145
73 and *S. lividans* TK21 [11].

74

75 By comparing the genome of *S. coelicolor* M145 to the five wild type genomes by using DNA
76 microarrays, we identified 22 sets of contiguous genes in *S. coelicolor* M145 that are absent in
77 the wild type strains [10]. We designated these genes Genomic Islands (GIs) 1 to 22. Fig. S1, Fig.
78 S1, and Fig. S3 show the GIs. The sizes of the GIs range from 3 kb to 150 kb and the number of
79 ORFs within them from 3 to 148. *S. coelicolor* M145 likely acquired the 22 GIs by horizontal

80 gene transfer, because they have the following characteristics: Direct repeats flank most of the
81 GIs; the GIs contain regions with low G + C content; the GIs encode transposable elements such
82 as invertases, recombinases, and transposons, as well as plasmid-related proteins; some GIs
83 appear to have inserted into tRNA sequences; and the GIs have slightly lower gene expression
84 during exponential growth [10,11]. We found that different wild type strains lack different GIs
85 and have different boundaries at the sites corresponding to the GIs in *S. coelicolor* M145. We
86 also identified DNA that the wild type strains possess and that *S. coelicolor* M145 lacks. Some of
87 that DNA (corresponding to GIs 8, 14, 17, 18, and 19 in *S. coelicolor* M145) might be large
88 segments, because we were unable to amplify the DNA by PCR [10]. The genomic islands have a
89 relatively uniform distribution across the chromosome of *S. coelicolor* M145, with a slight
90 abundance in the right chromosome arm [10,11].

91

92 A sigma factor is a specialized unit of RNA polymerase that permits the multisubunit enzyme to
93 initiate transcription selectively. Extracytoplasmic function (ECF) sigma factors constitute the
94 most abundant, smallest, and most divergent group of sigma factors [12]. Their name derives
95 from many of their members having roles in sensing and responding to signals generated
96 outside of the cell or in the cell membrane [13]. The genome of *S. coelicolor* M145 encodes 63
97 sigma factors, of which 49 belong to the ECF group [3]. During the early 2000's, we sought to
98 identify regulons of *S. coelicolor* sigma factors by overexpressing each one individually and
99 analyzing the response of the transcriptome by using DNA microarrays. The ECF sigma factor
100 encoded by SCO3450 was evaluated as part of that work. While we were unaware of this fact at
101 that time, SCO3450 is located in GI 6 [10].

102

103 The ECF sigma factor encoded by SCO3450 belongs to the subgroup ECF01 as classified by
104 Thorsten Mascher and colleagues in 2009 [14]. This subgroup includes RpoE-like sigma factors,
105 of which the best studied is σ^W of *Bacillus subtilis*. Members of the ECF01 subgroup are widely
106 distributed throughout bacterial phyla, but are absent in most actinobacteria, the phylum
107 which includes the genus *Streptomyces* [15]. ECF01 sigma factors have been characterized
108 experimentally to be involved in responses to envelope stress and the production and
109 detoxification of antimicrobial compounds [14].

110

111 **Results and Discussion**

112 *Genomic Island 6 encodes an ECF sigma factor and the regulon of the sigma factor*

113 Table 1 lists the five wild type *Streptomyces* strains that we studied that lack GI 6 [10].

114

115 Fig. 1 shows the boundaries of GI 6 (the left-hand columns) and the levels of transcripts, as
116 measured by microarrays, after artificial induction of SCO3450 in the strain *S. coelicolor* M600
117 growing exponentially in a liquid culture (the right-hand columns) [16]. Fifteen minutes after we
118 induced the sigma factor, transcripts of approximately one-fourth of the genes in GI 6 increased
119 in abundance. The genes numbered ~26: SCO3437 and SCO3442-3465; SCO3478 is an induced
120 gene located 14 kb away from the other genes. Cross-hybridization does not explain the result
121 for SCO3478 because the gene, which encodes a dehydrogenase, lacks sequence similarity to

122 other *S. coelicolor* genes. SCO3450 is located among the induced genes (Fig. 1, arrow; Fig. S4).
123 Together these data suggest that the ECF sigma factor and its regulon are a self-contained
124 transcriptional unit that can be transferred by horizontal gene transfer.

125

126 To our knowledge, the only other example of an ECF sigma factor and its regulon appearing to
127 be transferrable by horizontal gene transfer is the system for cobalt and nickel resistance in the
128 bacterium *Cupriavidus metallidurans* CH34 [18,19]. There, the plasmid pMOL28 contains six *cnr*
129 genes that are organized in two adjacent operons of three genes each. The gene *cnrH* encodes
130 the ECF sigma factor CnrH. Deletion and complementation of *cnrH* indicated that CnrH is
131 essential for the regulation of the *cnr* system. When CnrH was present but the periplasmic
132 sensor CnrX and the anti-sigma factor CnrY were absent, high-level constitutive expression was
133 observed for the *cnr* genes [18].

134

135 *The regulon of the ECF sigma factor contains conserved putative -35 and -10 promoter regions*

136 Byung-Kwan Cho and colleagues reported in 2016 transcription start sites of *S. coelicolor* genes
137 induced by 44 growth conditions [20]. Those conditions included growth in rich media and
138 many kinds of minimal media, growth in the liquid phase and on solid media, rapid growth to
139 stationary phase, and several kinds of shocks. That study did not identify transcription start
140 sites for the regulon of the ECF sigma factor encoded by SCO3450. This observation indicates
141 that the regulon is not induced by the growth conditions examined by Jeong et al. [20]. Perhaps

142 *S. coelicolor* uses the regulon for circumstances that are not replicated in the laboratory, such
143 as interactions in the natural environment among different microbial species.

144

145 We used the bioinformatics tool PromoterHunter [21], combined with visual inspection of
146 nucleotide sequences, to identify possible -35 and -10 promoter regions in the regulon of the
147 ECF sigma factor. The conserved sequences for the -35 and -10 promoter regions might be
148 AACGG and CG, respectively (Fig. 2). The promoters of 14 genes contain these sequences
149 exactly. The data suggest that operons might comprise SCO3445 to SCO3444, SCO3448 to
150 SCO3446, SCO3455 to SCO3452, and SCO3463 to SCO3465 (see Fig. S4). Alternatively, genes not
151 listed in Fig. 2 might have promoters with less conserved -35 and -10 regions. Note that
152 SCO3460 and SCO3478 do not possess the highly conserved promoter regions (see Fig. S4). In
153 addition, that SCO3438 has the conserved promoter regions supports the notion that this gene
154 belongs to the regulon of the ECF sigma factor. In Fig. 1, the lack of data for SCO3438 from all of
155 the microarray hybridizations indicates that its spot on the microarrays was functioning poorly.

156

157 *The regulon of the ECF sigma factor encodes putative enzymes for small molecule metabolism*

158 Recently we updated the annotations of the regulon of the ECF sigma factor by using
159 EnsemblBacteria [22] and UniProt BLASTS [23]. Of the approximately 26 genes in the regulon,
160 15 encode putative enzymes for small molecule metabolism. 11 genes encode putative
161 membrane and transport proteins. Four genes encode putative regulatory proteins. Four of the

162 26 genes fall into two categories. Only one gene lacks a putative function. Table S1 lists the
163 annotations of each gene in the regulon.

164

165 *Enzymes encoded by the regulon of the ECF sigma factor*

166 Table 2 lists the types of enzymes encoded by the regulon of the ECF sigma factor. The table
167 excludes SCO3461 and SCO3462, because they lack gene expression data (Fig. 1, right-hand
168 columns).

169

170 The putative enzymes fall into three general groups. One group possibly catalyzes the cleavage
171 of small molecules. The group includes a glycoside hydrolase, a nucleoside phosphorylase, and
172 a haloacid dehalogenase. A second group of enzymes might catalyze oxidation and reduction
173 reactions. The group includes a glutaredoxin-like protein, two oxidoreductases, and a
174 dehydrogenase. The third group of enzymes might catalyze transfer reactions. The group
175 includes two methyltransferases, perhaps a phosphatidyltransferase, and two nucleotide-
176 diphospho sugar transferases. A final gene, SCO3445, encodes a small membrane protein that
177 has 30-50% identity to small regions of seven larger proteins, a set which includes several
178 enzymes.

179

180 The composition of the enzymes in the regulon of the ECF sigma factor suggests that the
181 regulon might help to metabolize small molecules. A mixture of compounds might be being

182 degraded: sugars, nucleosides and nucleotides, and lipids for the purposes of energy and
183 biosynthesis; halogenated molecules for detoxification. Because the regulon encodes a set of
184 putative enzymes with diverse substrates, the small molecules might be coming from the
185 environment, for example, from neighboring cellular compartments or hyphae that are lysing
186 due to hostile conditions.

187

188 Haloacid dehalogenases (HADs) belong to a large superfamily of hydrolases with diverse
189 substrate specificity [24]. Type II HADs catalyze the hydrolytic dehalogenation of small L-2-
190 haloalkanoic acids to yield the corresponding D-2-hydroxyalkanoic acids [25]. Because many
191 *Streptomyces* bacteria produce halogenated antibiotics [26,27], the dehalogenase encoded by
192 SCO3446 might serve to defend *S. coelicolor* against competitors in the environment by helping
193 to catabolize antibiotics.

194

195 The following pairs of genes have no significant similarity, such that no cross-hybridization on
196 the microarrays should have occurred: SCO3452 and SCO3449, which encode the
197 methyltransferases; SCO3443 and SCO3460, which encode the oxidoreductases; and SCO3464
198 and SCO3465, which encode the nucleotide-diphospho-sugar transferases (Table 2).

199

200

201 *Anti-sigma factors within the regulon of the ECF sigma factor*

202 Two genes that possibly encode anti-sigma factors flank SCO3450, the gene encoding the ECF
203 sigma factor. The gene product of SCO3451 encodes a 103-amino-acid protein. A UniProt BLAST
204 shows that the protein has approximately 40% identity with varying coverages to putative anti-
205 sigma factors of other bacterial species, including putative transmembrane anti-sigma factors.
206 The Constrained Consensus TOPology (CCTOP) prediction server [28] predicts with a reliability
207 of 95.9069 that SCO3451 encodes a protein with one transmembrane segment (Fig. S5). The
208 gene product of SCO3451 also contains a putative zinc-finger found in some anti-sigma factor
209 proteins (Fig. S6). This zinc finger domain overlaps with the predicted transmembrane domain.

210

211 SCO3449 encodes a 106-amino-acid protein. A UniProt BLAST shows that the protein has
212 approximately 40% identity with coverages around 50% to putative anti-sigma factors in other
213 bacterial species, including putative transmembrane anti-sigma factors. However, according to
214 CCTOP, the protein does not contain a transmembrane domain. Like the putative anti-sigma
215 factor encoded by SCO3451, the putative anti-sigma factor encoded by SCO3449 contains a
216 possible zinc-finger found in some anti-sigma factor proteins (Fig. S7).

217

218 Microarray data are lacking for the expression of SCO3449 (Fig. 1, right-hand columns), such
219 that inferences about the role of this gene in comparison to those of the induced genes should
220 be made with caution. However, because microarray data are available for this gene in the
221 hybridizations that identified the genomic islands (Fig. 1, left-hand columns), the spot on the

222 microarrays that represented this gene was likely intact. It is possible that a low abundance of
223 transcripts of SCO3449 in both the reference and experimental samples of mRNA produced
224 poor signals from the microarray spot of the gene.

225

226 *Membrane and transport proteins encoded by the regulon of the ECF sigma factor*

227 Four contiguous genes in the regulon of the ECF sigma factor, SCO3453 to SCO3456, encode
228 proteins that likely constitute an ABC transporter system. UniProt BLASTS show that all of the
229 proteins have 45-50% identity with coverages greater than 95% to homologs with putative
230 functions in spermidine and putrescine transport in other bacterial species. In particular, the
231 species include *Geodermatophilus* and *Wenxinia*. Spermidine is a polyamine involved in cellular
232 metabolism that can be used to stimulate RNA polymerase. Putrescine attacks S-adenosyl
233 methionine and converts it to spermidine [29].

234

235 The putative transporters Sco3454 and Sco3455 have 34% identity with each other, the
236 coverage being 44% between the C-terminal portions of the proteins.

237

238 *Genomic Island 6 consists of four segments with transposases at their boundaries*

239 In addition to the regulon of the ECF sigma factor, we updated the annotations of the other
240 genes of Genomic Island 6 by using EnsemblBacteria and UniProt BLASTS (Table S2). In the
241 table, the location of the regulon is denoted by the words “REGULON HERE.”

242

243 The length of GI 6 is 108 kb. The genomic island consists of four segments of DNA bounded by
244 transposases (Table S2, pink color). Three of the four segments have coherent putative
245 functions: the oxidation and reduction of copper (Table S2, blue color); a characterized agarase
246 encoded by *dagA* [30,31]; and the utilization of sugars (Table S2, yellow color). Included in the
247 latter segment are a putative *lacI*-family transcriptional regulator and a putative β-
248 galactosidase. A large fourth segment encodes putative enzymes and many hypothetical
249 proteins.

250

251 The coherent functions of at least three of the four segments of GI 6 indicate that significant
252 portions of the island might be "active." If so, the regulon of the ECF sigma factor is likely active
253 as well. While a nucleotide blast of the regulon of the ECF sigma factor yielded no similar
254 segments of DNA in other sequenced organisms, the value to *S. coelicolor* of the regulon for as-
255 yet unknown reasons is indicated by the presence of the regulon on a genomic island. It would
256 be interesting to determine how common the regulon is among natural populations of
257 microbes and whether the regulon moves easily between hosts.

258

259

260 **Materials and Methods**

261 *Construction of a* S. coelicolor *strain that overexpresses SCO3450*

262 SCO3450, the gene which encodes the ECF sigma factor harbored by GI 6, was amplified by PCR
263 and cloned into the conjugative plasmid pIJ6902 [32] by using the restriction sites *Nde*I and
264 *Bgl*II. These restriction sites placed the gene immediately downstream of the thiostrepton-
265 inducible promoter *tipA*p. The resulting plasmid was transformed into the methylation-
266 deficient strain ET12567 with a non-transmissable helper plasmid, pUZ8002, and conjugated
267 into *S. coelicolor* M600 as described by Kieser et al. [33]. The exconjugants were selected by an
268 overlay of 50 μg/mL of apramycin.

269

270 *Time course of the overexpression strain*

271 Supplemented minimal medium (SMM) was inoculated with approximately 5 x 10^7 spores/mL
272 of a spore stock. The culture was grown at 30°C to early exponential phase, which
273 corresponded to a cell density of OD_{450} ~ 0.5. A reference sample, designated "0 min," was
274 harvested. To induce transcription of SCO3450, thiostrepton was added to the culture to a final
275 concentration of 30 μg/mL. Samples of the culture were harvested 15, 30, 45, 60, and 90
276 minutes after induction of SCO3450.

277

278

279 *Extraction of total RNA from the overexpression strain*

280 Samples of cells from liquid cultures were recovered by filtration on Whatman filter paper (15-
281 20 mm diameter; catalog #1002 055). RNA was isolated using the modified Kirby mix protocol
282 as described previously [34] with the following modifications: Harvested volumes ranged
283 between 5 mL and 20 mL, depending on the cell densities. RNA samples were treated only once
284 with DNase I (50-70 units; RNase-free, Invitrogen) for 15 minutes at room temperature.

285

286 *DNA microarray experiments for the overexpression strain*

287 Samples of cDNA were synthesized from total RNA as described previously [34]. cDNA of the
288 reference sample was labeled with Cy3-CTP. cDNAs of the samples isolated at subsequent time
289 points were labeled with Cy5-CTP. The cDNAs were hybridized to microarrays as described
290 previously [34].

291

292 *Identification of -35 and -10 conserved promoter regions in the regulon of the ECF sigma factor*

293 The tool PromoterHunter [21] was used to examine DNA upstream of the genes in the regulon
294 of the ECF sigma factor. Weight matrices corresponding to AAC for the -35 promoter region and
295 CG for the -10 promoter region were used, because they reflect the conserved promoter
296 regions of the subgroup ECF01 of ECF sigma factors [14]. A global G + C content of 72% was
297 used. The space between the -35 and -10 regions was specified to be between 17 to 21 base
298 pairs. Initially DNA segments of 300 base pairs were examined for isolated genes and genes

299 located at the beginning of likely operons. Visual inspection of sequences returned by
300 PromoterHunter identified AACGG and CG as possible consensus sequences for the -35 and -10
301 promoter regions, respectively. PromoterHunter was used to search for these conserved
302 sequences within DNA segments of 1000 base pairs upstream of all of the genes in the regulon,
303 in order to ensure that all instances of the sequences were identified.

304

305 **Acknowledgements**

306 We thank Thomas M. Privalsky for informing us about bioinformatic tools and Mark J. Buttner
307 and David A. Hopwood for discussions about the data.

308

309 **Author Contributions**

310 C.M.K. conceived the experiments. D.W. designed the experiments that compared the genomes
311 of the six *Streptomyces* strains and performed those experiments with J.A.V., M.L.H., and K.G.P.
312 N.K. designed the experiment overexpressing the ECF sigma factor and performed the
313 experiment with S.A.G. C.M.K. conducted the recent bioinformatic analyses and wrote the
314 manuscript.

315

316 **References**

317 1. Hopwood DA. 2007. Streptomyces in nature and medicine: the antibiotic makers. Oxford
318 University Press, New York, NY.

319 2. Kutzner HJ, Waksman SA. 1959. Streptomyces coelicolor Mueller and Streptomyces
320 violaceoruber Waksman and Curtis, two distinctly different organisms. J Bacteriol
321 78:528—538.
322 3. Bentley SD, Chater KF, Cerdeño-Tárraga AM, Challis GL, Thomson NR, James KD, Harris
323 DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M,
324 Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke
325 L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter
326 S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A,
327 Woodward J, Barrell BG, Parkhill J, Hopwood DA. 2002. Complete genome sequence of
328 the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141—147.
329 4. Hopwood DA. 1999. Forty years of genetics with *Streptomyces*: from *in vivo* through *in*
330 *vitro* to *in silico*. Microbiology 145:2183—2202.
331 5. Sermonti G, Spada-Sermonti I. 1955. Genetic recombination in *Streptomyces*. Nature
332 176:121.
333 6. Bradley SG. 1960. Reciprocal crosses in Streptomyces coelicolor. Genetics 45:613—619.
334 7. Lomovskaya ND, Mkrtumian NM, Gostimskaya NL, Danilenko VN. 1972. Characterization
335 of temperate actinophage phi C31 isolated from Streptomyces coelicolor A3(2). J Virol
336 9:258—262.
337 8. Kieser T, Hopwood DA, Wright HM, Thompson CJ. 1982. pIJ101, a multi-copy broad host-
338 range Streptomyces plasmid: functional analysis and development of DNA cloning
339 vectors. Mol Gen Genet 185:223—228.

340 9. Aguilar A, Hopwood DA. 1982. Determination of methylenomycin A synthesis by the
341 pSV1 plasmid from *Streptomyces violaceus-ruber* SANK 95570. J Gen Microbiol
342 128:1893—901.
343 10. Weaver D. 2005. PhD thesis. Stanford University, Stanford, CA. Genome plasticity in
344 *Streptomyces coelicolor*.
345 11. Jayapal KP, Lian W, Glod F, Sherman DH, Hu WS. 2007. Comparative genomic
346 hybridizations reveal absence of large *Streptomyces coelicolor* genomic islands in
347 *Streptomyces lividans*. BMC Genomics 8:229.
348 12. Feklístov A, Sharon BD, Darst SA, Gross CA. 2014. Bacterial sigma factors: a historical,
349 structural, and genomic perspective. Annu Rev Microbiol 68:357—376.
350 13. Lonetto MA, Brown KL, Rudd KE, Buttner MJ. 1994. Analysis of the Streptomyces
351 coelicolor sigE gene reveals the existence of a subfamily of eubacterial RNA polymerase
352 sigma factors involved in the regulation of extracytoplasmic functions. Proc Natl Acad
353 Sci U S A 91:7573—7577.
354 14. Staroń A, Sofia HJ, Dietrich S, Ulrich LE, Liesegang H, Mascher T. 2009. The third pillar of
355 bacterial signal transduction: classification of the extracytoplasmic function (ECF) σ
356 factor protein family. Mol Microbiol 74:557—581.
357 15. ECFfinder. http://ecf.g2l.bio.uni-goettingen.de:8080/ECFfinder/.
358 16. Takano E, White J, Thompson CJ, Bibb MJ. 1995. Construction of thiostrepton-inducible,
359 high-copy-number expression vectors for use in *Streptomyces* spp. Gene 166:133—137.

360 17. Huang J, Lih CJ, Pan KH, Cohen SN. 2001. Global analysis of growth phase responsive
361 gene expression and regulation of antibiotic biosynthetic pathways in *Streptomyces*
362 *coelicolor* using DNA microarrays. Genes Dev 15:3183—3192.
363 18. Grass G, Grosse C, Nies DH. 2000. Regulation of the *cnr* cobalt and nickel resistance
364 determinant from *Ralstonia* sp. strain CH34. J Bacteriol 182:1390—1398.
365 19. Tibazarwa C, Wuertz S, Mergeay M, Wyns L, van der Lelie D. 2000. Regulation of the *cnr*
366 cobalt and nickel resistance determinant of *Ralstonia eutropha* (*Alcaligenes eutrophus*)
367 CH34. J Bacteriol 182:1399—1409.
368 20. Jeong Y, Kim JN, Kim MW, Bucca G, Cho S, Yoon YJ, Kim BG, Roe JH, Kim SC, Smith CP,
369 Cho BK. 2016. The dynamic transcriptional and translational landscape of the model
370 antibiotic producer *Streptomyces coelicolor* A3(2). Nat Commun 7:11605.
371 21. Klucar L, Stano M, Hajduk M. 2010. phiSITE: database of gene regulation in
372 bacteriophages. Nucleic Acids Res 38:D366—D370.
373 22. Kersey PJ, Allen JE, Allot A, Barba M, Boddu S, Bolt BJ, Carvalho-Silva D, Christensen M,
374 Davis P, Grabmueller C, Kumar N, Liu Z, Maurel T, Moore B, McDowall MD, Maheswari
375 U, Naamati G, Newman V, Ong CK, Paulini M, Pedro H, Perry E, Russell M, Sparrow H,
376 Tapanari E, Taylor K, Vullo A, Williams G, Zadissia A, Olson A, Stein J, Wei S, Tello-Ruiz M,
377 Ware D, Luciani A, Potter S, Finn RD, Urban M, Hammond-Kosack KE, Bolser DM, De
378 Silva N, Howe KL, Langridge N, Maslen G, Staines DM, Yates A. 2018. Ensembl Genomes
379 2018: an integrated omics infrastructure for non-vertebrate species. Nucleic Acids Res
380 46:D802—D808.

381 23. The UniProt Consortium. 2017. UniProt: the universal protein knowledgebase. Nucleic
382 Acids Res 45:D158—D169.
383 24. Koonin EV, Tatusov RL. 1994. Computer analysis of bacterial haloacid dehalogenases
384 defines a large superfamily of hydrolases with diverse specificity: application of an
385 iterative approach to database search. J Mol Biol 244:125—132.
386 25. Janssen DB, Oppentocht JE, Poelarends GJ. 2001. Microbial dehalogenation. Curr Opin
387 Biotechnol 12:254—258.
388 26. van Pée KH. 1996. Biosynthesis of halogenated metabolites by bacteria. Annu Rev
389 Microbiol 50:375—399.
390 27. Rezanka T, Spizek J. 2003. Halogen-containing antibiotics from Streptomycetes, p 309—
391 353. *In* Atta-ur-Rahman (ed), Studies in natural products chemistry, 1st ed, vol 29.
392 Elsevier Science.
393 28. Dobson L, Reményi R, Tusnády GE. 2015. CCTOP: a Consensus Constrained TOPology
394 prediction web server. Nucleic Acids Res 43:W408—W412.
395 29. Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Chang HY, Dosztányi Z,
396 El-Gebali S, Fraser M, Gough J, Haft D, Holliday GL, Huang H, Huang X, Letunic I, Lopez R,
397 Lu S, Marchler-Bauer A, Mi H, Mistry J, Natale DA, Necci M, Nuka G, Orengo CA, Park Y,
398 Pesseat S, Piovesan D, Potter SC, Rawlings ND, Redaschi N, Richardson L, Rivoire C,
399 Sangrador-Vegas A, Sigrist C, Sillitoe I, Smithers B, Squizzato S, Sutton G, Thanki N,
400 Thomas PD, Tosatto SC, Wu CH, Xenarios I, Yeh LS, Young SY, Mitchell AL. 2017. InterPro
401 in 2017--beyond protein family and domain annotations. Nucleic Acids Res 45:D190—
402 D199.

403 30. Temuujin U, Chi WJ, Lee SY, Chang YK, Hong SK. 2011. Overexpression and biochemical
404 characterization of DagA from *Streptomyces coelicolor* A3(2): an endo-type β-agarase
405 producing neoagarotetraose and neoagarohexaose. Appl Microbiol Biotechnol 92:749—
406 759.
407 31. Bibb MJ, Jones GH, Joseph R, Buttner MJ, Ward JM. 1987. The agarase gene (*dagA*) of
408 *Streptomyces coelicolor* A3(2): affinity purification and characterization of the cloned
409 gene product. J Gen Microbiol 133:2089—2096.
410 32. Huang J, Shi J, Molle V, Sohlberg B, Weaver D, Bibb MJ, Karoonuthaisiri N, Lih CJ, Kao
411 CM, Buttner MJ, Cohen SN. 2005. Cross-regulation among disparate antibiotic
412 biosynthetic pathways of *Streptomyces coelicolor*. Mol Microbiol 58:1276—1287.
413 33. Kieser T, Bibb MJ, Buttner, MJ, Chater KF, Hopwood DA. 2000. Practical *Streptomyces*
414 genetics. John Innes Foundation.
415 34. Weaver D, Karoonuthaisiri N, Tsai HH, Huang CH, Ho ML, Gai S, Patel KG, Huang J, Cohen
416 SN, Hopwood DA, Chen CW, Kao CM. 2004. Genome plasticity in *Streptomyces*:
417 identification of 1 Mb TIRs in the *S. coelicolor* A3(2) chromosome. Mol Microbiol
418 51:1535—1550.

419

420 **FIG 1** Genomic Island 6 and the regulon of the ECF sigma factor encoded by SCO3450. GI 6 is
421 108 kb in length. The arrow indicates the location of SCO3450, which encodes a putative ECF
422 sigma factor. See Table 1 for a list of the strains used to identify GI 6. To obtain the data in the
423 figure, we used DNA microarrays that contained the 7825 predicted genes in the chromosome
424 of *S. coelicolor* M145 [17]. For the left-hand columns, the maximum intensity of green

425 corresponds to a 3-fold difference in signal on the microarrays for DNA in *S. coelicolor* M145
426 relative to DNA in the wild type strains. SCO3450 was overexpressed in an exponentially
427 growing liquid culture (SMM) of a *S. coelicolor* M600 derivative, through the use of a
428 thiostrepton-inducible promoter [16]. RNA was harvested from the culture immediately prior to
429 the addition of thiostrepton and every 15 to 30 minutes afterwards for 90 minutes. cDNA of the
430 initial RNA sample (OD_{450} ~ 0.5) was labeled with the green fluorescent dye Cy3. cDNA of RNA
431 isolated from subsequent time points was labeled with the red fluorescent dye Cy5. Yellow
432 microarray spots for a particular time point represented genes with transcript levels equal to
433 the levels of the initial time point. Red and green microarray spots represented genes induced
434 and repressed, respectively, after the addition of thiostrepton. The yellow color is shown here
435 as black for clarity. For the right-hand columns, the maximum intensity of red corresponds to 8-
436 fold induction of a given gene at a particular time relative to the beginning of the time course. A
437 control experiment with a M600 derivative that lacks SCO3450 in the induction plasmid
438 identified genes induced by thiostrepton. Labels on the right indicate ORF numbers and genes
439 with names.

440

441 **FIG 2** Alignment of putative promoters in the regulon of the ECF sigma factor encoded by
442 SCO3450. Red letters indicate the putative -35 and -10 promoter regions. On the right-hand
443 side, the numbers in parentheses indicate the distance between each -10 promoter region and
444 the start codon of the respective gene.

445

446

447 **Table 1. Strains that revealed the genomic islands of *S. coelicolor* M145.**

Strain	Comment	Ref.
M145	*S. coelicolor* sequenced “reference” strain, SCP1- SCP2-	[3]
1152	*S. coelicolor* Sermonti's SE1	[5]
1153	*S. coelicolor* Bradley's S199 strain	[6]
1326	*S. lividans* 66	[7]
2896	*S. lividans* ISP5434-, the strain from which pIJ101 was isolated	[8]
3034	*S. violaceroruber* SANK95570, harbors pSV1, which encodes methylenomycin biosynthetic genes	[9]

448

449

450 **Table 2. Types of enzymes encoded by the regulon of the ECF sigma factor.**

Type	Gene	AA	Putative Function	Other feature
CLEAVAGE				
Glycoside hydrolase	SCO3444	617	glycoside hydrolase family 15/phosphorylase b kinase regulatory chain family; six-hairpin glycosidase	
Nucleoside phosphorylase	SCO3463	262	nucleoside phosphorylase domain	
Dehalogenase	SCO3446	225	haloacid dehydrogenase (HAD)-like domain	
REDOX				
Glutaredoxin	SCO3442	114	glutaredoxin (DNA synthesis?)	
Oxidoreductase	SCO3443	454	pyridine nucleotide-disulphide oxidoreductase; dihydrolipoyl dehydrogenase?	
	SCO3460	505	pyridine nucleotide-disulphide oxidoreductase; dihydrolipoamide dehydrogenase?	
Dehydrogenase	SCO3478	344	D-isomer specific 2-hydroxyacid dehydrogenase	
TRANSFER				
Methyltransferase	SCO3452	359	S-adenosyl-L-methionine-dependent methyltransferase	
	SCO3459	287	S-adenosyl-L-methionine-dependent methyltransferase	
Phosphatidyltransferase?	SCO3457	205	CDP-alcohol phosphatidyltransferase; YnjF?	transmembrane
Nucleotide-diphospho-sugar transferase	SCO3464	210	nucleotide-diphospho-sugar transferases; transferase 1, rSAM/selenodomain-associated	
	SCO3465	236	nucleotide-diphospho-sugar transferase; transferase 2, rSAM/selenodomain-associated	
MISC				
Enzyme?	SCO3445	55	low to moderate homology to small regions of seven larger proteins, which include enzymes	membrane

451 AA = amino acids

452

453

454 **Legends for Supplemental Material**

455 **FIG S1** Genomic Islands 1 to 10 of *S. coelicolor* M145. Only the genomic islands are shown and
456 not the entire chromosome. Table 1 lists the strains used. To obtain these data, we used DNA
457 microarrays that contained the 7825 predicted genes in the chromosome of *S. coelicolor* M145
458 [17]. Genomic DNA from *S. coelicolor* M145 was used as the reference sample and labeled with
459 the green fluorescent dye Cy3. Genomic DNA samples from the five wild type strains each were
460 labeled with the red fluorescent dye Cy5. The labeled DNA of each wild type strain was mixed
461 with the labeled DNA of *S. coelicolor* M145 and hybridized to a microarray. Yellow spots on the
462 microarrays represented genes at equal copy numbers between *S. coelicolor* M145 and the
463 other strains. Green spots represented genes present in *S. coelicolor* M145 but absent in the
464 other strains. Red spots represented genes at higher copy numbers in the strains relative to *S.*
465 *coelicolor* M145. The yellow color is shown here as black for clarity. The maximum intensity of
466 green corresponds to a 3-fold difference in signal on the microarrays for DNA in *S. coelicolor*
467 M145 relative to DNA in the wild type strains. The green bars to the left of the GIs designate
468 horizontally transferred genes in the genome sequence of *S. coelicolor* M145 that were
469 predicted by Bentley et al. [3]. Regions of GIs not predicted by Bentley et al. lack a bar. Labels
470 on the right indicate ORF numbers and genes with names.

471

472 **FIG S2** Genomic Islands 11 to 18 of *S. coelicolor* M145. See the text of Fig. S1.

473

474 **FIG S3** Genomic Islands 19 to 22 of *S. coelicolor* M145. See the text of Fig. S1.

475

476 **FIG S4** The region of the *S. coelicolor* M145 chromosome that contains the regulon of the ECF

477 sigma factor. The figure was obtained from EnsemblBacteria [22]. SCO3478 is not shown.

478

479 **FIG S5** Result from CCTOP for the putative anti-sigma factor encoded by SCO3451. The putative

480 anti-sigma factor is predicted to contain one transmembrane domain. The data were obtained

481 from the Constrained Consensus TOPology (CCTOP) prediction server [28].

482

483 **FIG S6** Predicted zinc finger in the putative anti-sigma factor encoded by SCO3451. The data

484 were obtained from UniProt [23].

485

486 **FIG S7** Predicted zinc finger in the putative anti-sigma factor encoded by SCO3449. The data

487 were obtained from UniProt [23].

488

489 **Table S1. Annotations of the regulon of the ECF Sigma Factor encoded by SCO3450.**

490 Annotations were obtained from EnsemblBacteria [22] and UniProt [23]. For the UniProt

491 BLASTs, parentheses denote genera and percent identities of similar proteins. Red text denotes

492 putative enzymes. Blue text denotes proteins with homology to small regions of larger proteins.

493

494 **Table S2. Annotations of Genomic Island 6, excluding the regulon of the ECF sigma factor**

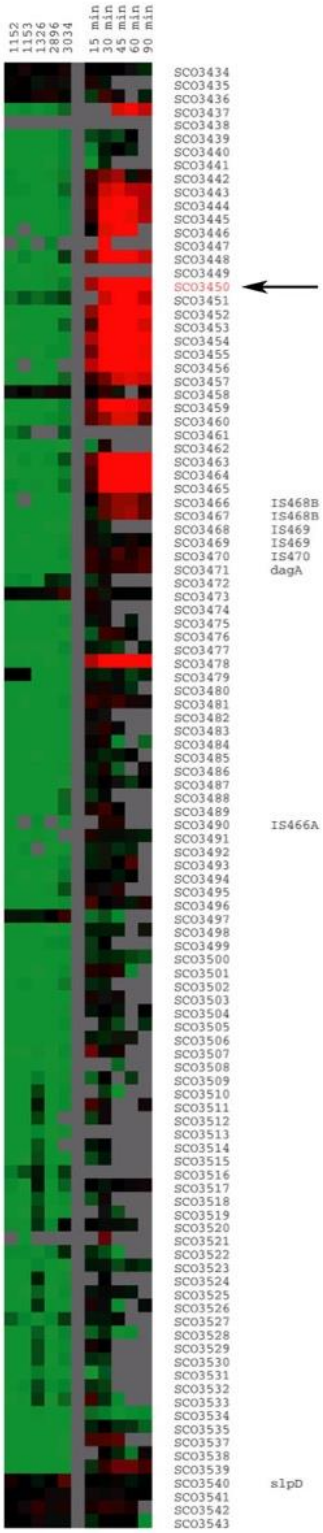
495 **encoded by SCO3450.** Annotations were obtained from EnsemblBacteria [22] and UniProt [23].

496 For the UniProt BLASTs, parentheses denote genera and percent identities of similar proteins.

497 Red text denotes putative enzymes. Blue text denotes proteins with homology to small regions

498 of larger proteins. See the text for additional details.

499



500

501 **FIG 1** Genomic Island 6 and the regulon of the ECF sigma factor encoded by SCO3450.

```
SCO3437-1 CGTTGCCGACAACGGGATGATGATCCTCGG--CGACCTCAACCA (694 bp)
SCO3437-2 CGTCGCCGACAACGGGATGATGATCCTCGG--CGACCTCAACCA (283 bp)
SCO3438   CAGGTCCCGCAACGGCGCACCCTCGTGGGC--CGGTGCCCGCGG (178 bp)
SCO3442   GTGGTGGTGGAACGGCACCTGGCTGCCGCAGTCCGTGCCGCCCG (407 bp)
SCO3443   CGACCAGGCCAACGGCGCGCTACCTCGCCACGACGCCGACCCGG (158 bp)
SCO3445   ACGGCCTGGAAACGGCGTGCTCCTGAGCCGC-CGTGCGTCAATG (  7 bp)
SCO3448   TCGATGGCGAAACGGACGAGGTCACCGCACGCCGGGTCGCGGCC (295 bp)
SCO3451   TTCCCGTCGAAACGGTCGATGGCCCGGTAGGCGCGCAGCAAGGT (312 bp)
SCO3455-1 GGTGTGGGTCAACGGAGAGAACTTCCGCAC--CGGAAAGCAGGC (883 bp)
SCO3455-2 CTGGACCAGGAACGGCTGCCCGAGCAGTGG--CGAGAGAAGTTC (150 bp)
SCO3456   GTTTCCGCGCAACGGATGTTCGCGGGACCGTCGCGCGGGAAACA ( 50 bp)
SCO3457   GTTGCGCGGAAACGGAACACGGCCGGGTGCT-CGCAGGCTTCCC ( 58 bp)
SCO3458   GCGTGTCCGAAACGGAATGCGTGACCAGCC--CGGCAGGGTTCC (844 bp)
SCO3459   GTCGCTGGTCAACGGCCCGCTCTCGACCAGGCCGGGGATCGGCA (611 bp)
SCO3462   CCGAGGCCGTAACGGCCCTCTGGTTTCTCGCCCGGCCCCGCTCC (276 bp)
SCO3463   CCACCCCGAGAACGGAACCCTCGTCCCCGCCTGCGTACAGCACG (145 bp)
                        -35                  -10
```

FIG 2 Alignment of putative promoters in the regulon of the ECF sigma factor encoded by SCO3450.