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Transcriptional Analysis of the *Streptomyces glaucescens* Tetracenomycin C Biosynthesis Gene Cluster

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A 12.6-kb DNA fragment from *Streptomyces glaucescens* GLA.0 containing the 12 genes for tetracenomycin (TCM) C biosynthesis and resistance enabled *Streptomyces lividans* to produce TCM C. Transcriptional analysis of the *tcmPG* intergenic region in this cluster established the presence of two divergent promoters. The *tcmIc* mutation, a T-to-G transversion in the −10 region of the *tcmG* promoter, decreased promoter activity drastically at the stationary growth stage and time of maximum TCM C accumulation. This promoter may direct the transcription of a *tcmGHIJKLMNO* operon, while the other promoter is for *tcmP*.

The study of biosynthesis of the anthracycline antibiotic tetracenomycin (TCM) C in *Streptomyces glaucescens* ETH 22794 (GLA.0) (40) is one of the current models for the genetics of secondary metabolite production in *Streptomyces* species, particularly for polyketide-derived metabolites (7). Sequence analysis of the entire TCM C gene cluster has been completed (2, 10, 15, 25, 37), and the products of different *tcm* genes are now undergoing biochemical characterization. Transcriptional analysis of this cluster is an important step towards understanding the regulation of TCM C biosynthesis. Guilfoile and Hutchinson have demonstrated that the *tcmA* resistance and *tcmR* repressor gene loci (Fig. 1) are divergently transcribed (15). Transcription of *tcmA* is induced by TCM C (16), and the TcmR protein binds to operator sites in the *tcmAR* intergenic region and represses transcription of both *tcmA* and *tcmR*.

The transcriptional organization of the 10 TCM C biosynthetic genes identified (Fig. 1) has not been studied previously, but the results of sequence analysis of the *tcm* cluster suggest that the *tcmP* gene, which is flanked downstream by the convergently transcribed *tcmA* gene (15) and upstream by the rest of the TCM C biosynthetic genes, has a monocistronic mRNA (10). A 438-bp gap separates the *tcmP* and *tcmG* loci (10). The genes downstream of *tcmG* are arranged in close proximity to each other and transcribed in the same direction (2, 10, 25, 37). Characterization of the *tcmP* and *tcmG* promoters by primer extension and S1 nuclease mapping experiments has now led to the discovery that the *tcmIc* mutation, a T-to-G transversion in the −10 region of the *tcmG* promoter (10), essentially abolishes *tcmG* promoter function at the time of maximum TCM C production. This could be the reason for the observed lack of function of the *tcmGHIJKLMNO* genes in the *tcmIc* mutant (24), if they in fact constitute an operon under control of the *tcmG* promoter.

Expression of the TCM C gene cluster in *Streptomyces lividans*. To determine the minimum number of genes for TCM C biosynthesis, we ligated a 12.6-kb *Not*I-*Bam*HI segment (Fig. 1) containing all the known TCM C biosynthetic genes as well as the *tcmAR* resistance and repressor genes (15) into the high-copy-number plasmid vector pWHM3 (39) as described in Table 1 (following the general cloning procedures of Decker et al. [10]) to give pWHM1026. When *S. lividans* JT46 (38) was transformed with pWHM1026 (see reference 10 for transformation procedures), TCM C was produced as confirmed by bioassay and thin-layer and high-performance liquid chromatography (10). The recombinant *S. lividans* strain produced approximately five times less TCM C than *S. glaucescens* GLA.0 when grown in R2YENG medium (10) to the stationary growth stage. Thus, the DNA between the region 383 bp downstream of the *tcmO* gene (37) and 31 bp downstream of the *tcmR* repressor gene (15) (Fig. 1) contains all of the genes required for TCM C production in a heterologous host.

Since only *tcmP* among the 10 *tcm* structural genes contains a TTA codon (10) and a *bldA* mutation apparently can prevent the expression of several *Streptomyces* genes having TTA codons (20, 21), we assayed the metabolites produced by an *S. lividans* J1725 *bldA*(pWHM1026) transformant by thin-layer chromatography and high-performance liquid chromatography. One possibility was that, in this background, TCM C biosynthesis would be blocked at the step where TCM A2 is made as a consequence of the action of the *tcmP* gene product and result in the accumulation of TCM E (10). However, TCM C was still produced by this transformant, albeit at a level six times less than that of the isogenic *S. lividans* 1326-9(pWHM1026) *bldA*$^+$ strain. TCM E, which was not detectable in the *S. lividans* 1326-9(pWHM1026) culture, was also produced by the transformed *bldA* mutant. Thus, *S. lividans* J1725 apparently can read the UUA triplet by the wobble process or mistranslation (20) well enough to permit a small amount of functional TcmP to be produced. We did not compare the specific activity of TcmP in the *bldA*$^+$ and *bldA* backgrounds but know that *S. lividans* in unable to *O*-methylate TCM E without the *tcmP* gene being present (10).

Analysis of the promoters of the *tcmP* and *tcmG* genes. Preliminary low-resolution S1 nuclease protection experiments with a 1,300-bp *tcmG* probe (Fig. 1, segment c) and a 1,030-bp *tcmP* probe (Fig. 1, segment b) established the approximate time of gene transcription and promoter location. Using RNA samples isolated (8) from 12.5-, 20-, and 25-h cultures of the GLA.0 strain, which represent, respectively, the exponential, transition to stationary, and early-to-mid-stationary stages of growth (the last point is when

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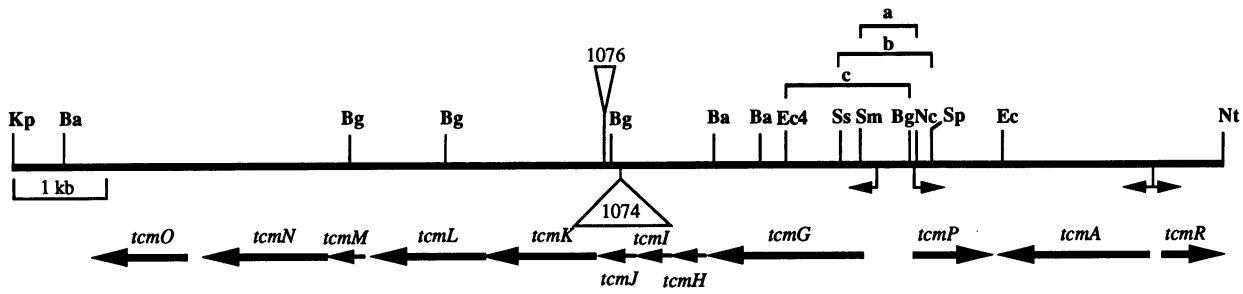

FIG. 1. Organization of the *tcm* gene cluster of *S. glaucescens*. The thick arrows beneath the restriction map indicate the location and direction of transcription of the 12 *tcm* genes. The bars above the map indicate three restriction fragments: a, the *Sma*I-*Nco*I segment that was used for promoter analysis; b and c, two segments used in low-resolution S1 mapping experiments to define the promoters of the *tcmG* and *tcmP* genes. The triangles above and below the map show the locations and approximate sizes of DNA insertions for the two *tcmIb* mutations discussed in the text. Abbreviations (only restriction sites of interest are shown): Ba, *Bam*HI; Bg, *Bgl*II; Ec, *Eco*RI; Ec4, *Eco*47III; Kp, *Kpn*I; Nc, *Nco*I; Nt, *Not*I; Sm, *Sma*I; Sp, *Sph*I; Ss, *Sst*I.

maximum production of TCM C occurs), we could identify S1 nuclease-protected fragments of 940 and 320 bp, respectively, for the *tcmG* and *tcmP* transcripts in RNA from wild-type cultures at the three ages tested. The amount of RNA from the 5′ end of *tcmG* increased considerably from 12.5 to 20 h and underwent an increasing amount of degradation between 20 and 25 h, as evidenced by the appearance of protected DNA of lower molecular weights (data not shown). No *tcmG* message was seen when the same experiment was performed with RNA isolated from the *tcmIc* mutant. Much less *tcmP* message was detected at the same time points, although its amount also increased from 12.5 to 20 h (data not shown).

TCM C was detected by bioautography and chromatography (10) in the 20- and 25-h cultures, as expected from earlier work (24, 37), but was also seen in the 12.5-h culture at a concentration 6 and 150 times lower than in the 20- and 25-h cultures, respectively. TCM C production was not detectable in the *tcmIc* mutant by the same method.

The *tcmG* and *tcmP* promoters were further characterized by primer extension and high-resolution S1 mapping experiments. The latter were performed as described by Neal and Chater (26), except that 6% polyacrylamide–8 M urea–10% formamide wedge gels were used and probe DNA was prepared as follows. Probes were synthesized by the method of Sharrocks and Hornby (32) with 24-mer oligomers (made with a model 391 DNA synthesizer; Applied Biosystems, Foster City, Calif.) as primers (primer 1, 5′-GCCTGAAGT GTGGCGAGCAGCGTC-3′; primer 2, 5′-GGGGATTTGC TCGGGCCGACGAGC-3′), except that [^{35}S]dCTP was used to label the DNA (1). The probes were subsequently purified on denaturing 8 M urea–3% polyacrylamide gels and eluted

TABLE 1. Plasmids used in this study

Plasmid	Construction and characteristics	Reference
pWHM1013	3.4-kb *Eco*RI-*Pst*I fragment in pWHM3; *tcmP tcmG*	10
pWHM1027	2.1-kb fragment containing the *S. glaucescens tcmIc* allele	10
pIJ2839	pXE3 digested with *Hin*dIII and *Bgl*II and ligated with the following polylinker: *Hin*dIII-*Sph*I-*Sal*I-*Xba*I-*Bam*HI-*Sma*I-*Kpn*I-*Sst*I-*Eco*RI-*Bgl*II; *Escherichia coli-Streptomyces* low-copy-number shuttle vector with *xylE* reporter gene	9, 19
pWHM1020	pWHM1008 (10) digested with *Nco*I and *Sma*I and the resulting 0.67-kb fragment ligated into the *Nco*I-EcoRV site of pGEM5zf(+) (Promega Madison, Wis.); intergenic region (Fig. 1, segment a)	This work
pWHM1021	pWHM1020 digested with *Sal*I and *Sph*I and the resulting 0.67-kb fragment ligated into the *Sal*I-*Sph*I site of pUC19 (41); intergenic region (Fig. 1, segment a)	This work
pWHM1022	pWHM1020 digested with *Pst*I-*Nco*I and the resulting 0.67-kb fragment treated with the Klenow fragment of DNA polymerase I and ligated into pUC19 that was digested with *Xba*I and *Pst*I and treated with the Klenow fragment; intergenic region (Fig. 1, segment a)	This work
pWHM1023	pWHM1021 digested with *Bam*HI and *Hin*dIII and the resulting 0.67-kb fragment ligated into the *Bam*HI-*Hin*dIII site of pIJ2839; *tcmG* promoter	This work
pWHM1024	pWHM1022 digested with *Bam*HI and *Hin*dIII and the resulting 0.67-kb fragment ligated into the *Bam*HI-*Hin*dIII site of pIJ2839; *tcmP* promoter	This work
pWHM1025	pWHM109 (23) digested with *Bam*HI and *Pst*I and the resulting 6.7-kb fragment ligated together with a 3.4-kb fragment from a *Pst*I-*Eco*RI digest of pWHM1013 into the *Bam*HI-*Pst*I site of pBluescript KS+ (Strategene, La Jolla, Calif.); *tcmPGHIJKLMNO*	This work
pWHM1026	pWHM1025 digested with *Xba*I and *Eco*RI and the resulting 10.1-kb fragment ligated together with a *Hin*dIII-*Eco*RI digest of pWHM634 (16) into the *Xba*I-*Hin*dIII site of pWHM3; *tcmARPGHIJKLMNO*	This work
pWHM1028	pWHM1027 digested with *Sac*II and *Bgl*II and ligated into the *Sac*II-*Bgl*II site of pWHM1021; construct sequenced to confirm the presence of the characteristic *tcmIc* mutation (10); *tcmG*^{d} promoter	This work
pWHM1029	pWHM1028 digested with *Bam*HI and *Hin*dIII and ligated into the *Bam*HI-*Hin*dIII site of pIJ2839; *tcmG*^{d} promoter	This work

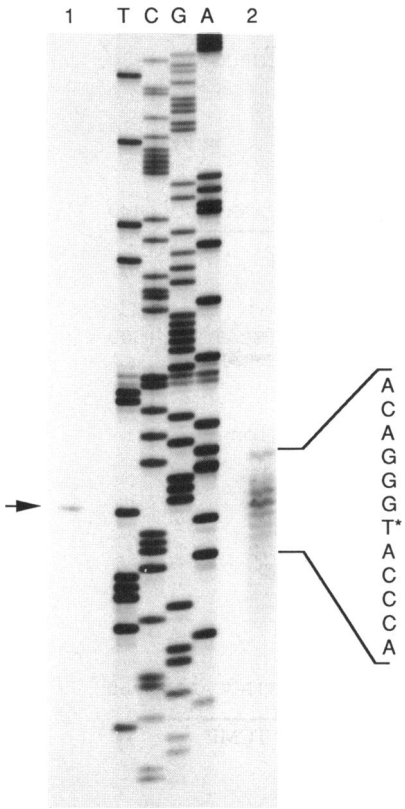

FIG. 2. Transcript mapping of the *tcmP* gene. Lane 1, DNA from primer extension analysis (the arrow indicates the position of the DNA fragment); lane 2, S1 nuclease-protected DNA; lanes T, C, G, and A, sequencing reactions generated with primer 1 (see Fig. 4B). The asterisk indicates the apparent transcriptional start point.

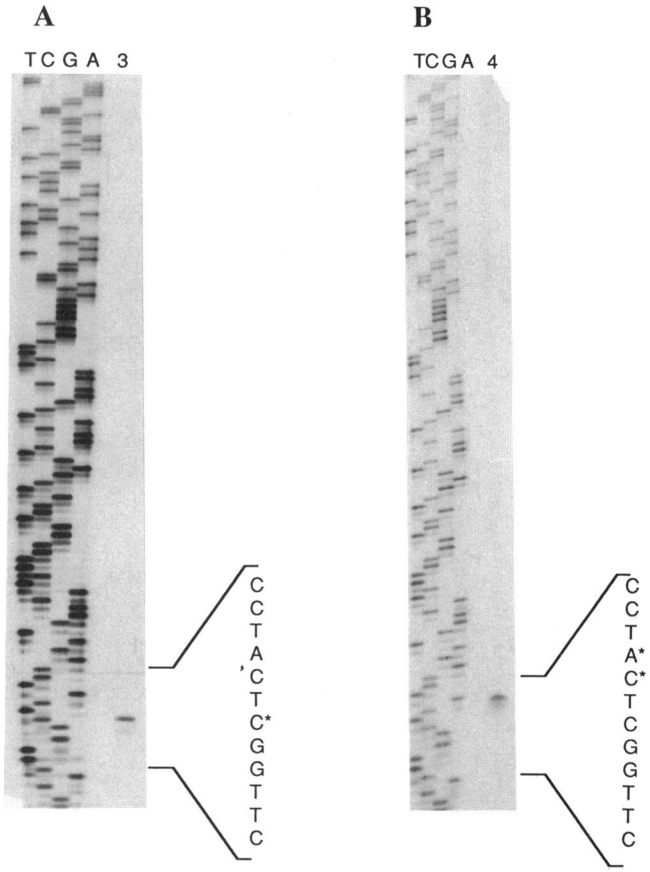

FIG. 3. Transcript mapping of the *tcmG* gene. (A) Lane 3, DNA from primer extension analysis; (B) lane 4, S1 nuclease-protected DNA. Lanes T, C, G, and A, sequencing reactions generated with primer 2 (see Fig. 4A). The asterisks indicate the apparent transcriptional start points. As stated in the text, nucleotide 1403 (G) is believed to be the true transcriptional start point, and the results obtained by S1 nuclease mapping are experimental artifacts giving rise to a transcript that is 2 to 3 nucleotides longer.

by using the crush and soak method (31). A sequencing reaction was done with the relevant DNA fragments to determine the size of the protected fragment and the transcriptional start site. Primer extension experiments were performed as described by Stein et al. (34) with the modifications described by Guilfoile and Hutchinson (15), using Superscript mouse mammary leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, Md.). Actinomycin D (50 μg/ml; Sigma) was added to the reverse transcriptase reaction to prevent synthesis of hairpin artifacts (31). Single apparent 5′ start points were obtained for the *tcmG* and *tcmP* genes in primer extension experiments with RNA taken from 25-h cultures (Fig. 2 and 3). High-resolution S1 mapping experiments confirmed the apparent transcriptional start point for both genes, although the bands obtained by this method are more heterogeneous (Fig. 2 and 3). It is likely that S1 nuclease does not cleave the probe exactly at the end of the DNA-RNA hybrid because it either is sterically inhibited (6) or cannot cleave at the thioester bonds of the probe (13). The apparent 5′ end of the *tcmP* transcript (Fig. 2) is located at nucleotide 966 in Fig. 2 of the accompanying paper (10) and is identical to the presumed translational start site of the *tcmP* gene. Transcription of *tcmG* appears to begin at nucleotide 1403. The sequences that precede the apparent transcriptional start points and the presumed locations of the deduced −35 and −10 regions for both promoters are illustrated in Fig. 4. These data show that the point mutation found in the *tcmIc* strain (10) is located in the putative −10 region of the *tcmG* promoter. We designate this promoter *tcmG*^{d}.

The *tcmP*, *tcmG*, and *tcmG*^{d} promoters were characterized further by cloning a 0.67-kb *Sma*I-*Nco*I fragment (Fig. 1, segment a) containing each of these promoters upstream of the *xylE* gene (43) in the low-copy-number vector pIJ2839 (9, 27) (Table 1). XylE activities (19, 30) were determined in crude cell extracts obtained from *S. glaucescens* transformed with pWHM1023 (*tcmG*::*xylE*), pWHM1029 (*tcmG*^{d}::*xylE*), and pWHM1024 (*tcmP*::*xylE*), using 24-h cultures grown in R2YENG medium (10) as follows. Samples (1 ml) were taken from liquid cultures, centrifuged, and washed twice with 20 mM potassium phosphate buffer, pH 7.5. The pellet was resuspended in 500 μl of 100 mM potassium phosphate buffer (pH 7.5)–10% acetone, the cells were disrupted by sonication, and the cell debris was removed by centrifugation at 13,000 × *g*. The XylE assay was performed in 100 mM potassium phosphate buffer, pH 7.5, with 1 mM catechol without acetone at 24°C, and the increase in A_{340} was monitored. Qualitative XylE assays were done by spraying agar plates with an aqueous solution of 0.2 M catechol. The protein content of assay mixtures was determined as described by Bradford (5) with bovine serum

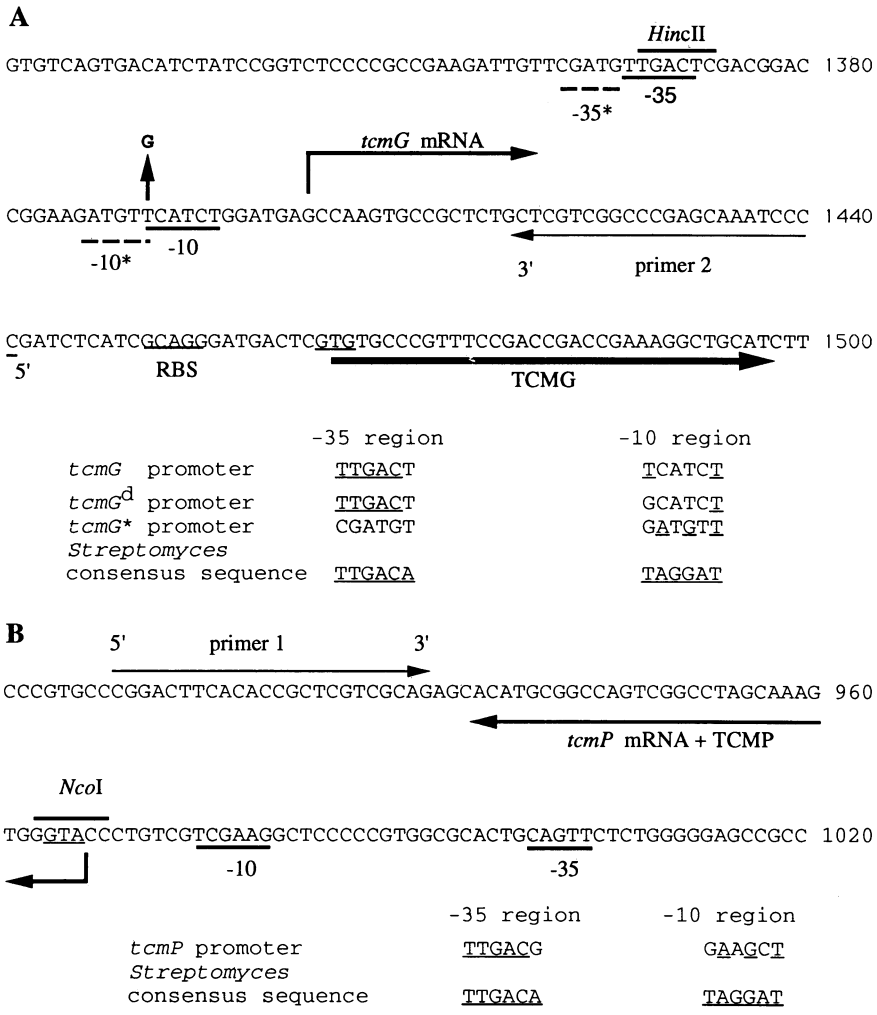

FIG. 4. (A) *tcmG* promoter sequence of *S. glaucescens*. *tcmG*, the sequence of the wild-type *tcmG* promoter; *tcmG*^{d}, promoter sequence of the *tcmIc* mutant; *tcmG**, alternative assignment of the *tcmG* promoter. Start points and direction of transcription and translation, as well as the location of primer 2, are indicated by arrows. The ribosome binding site is underlined, and the location of the base transversion in the promoter sequence of the *tcmIc* mutant (10) is indicated by a vertical arrow. (B) *tcmP* promoter sequence of *S. glaucescens* and its comparison with a *Streptomyces* consensus sequence. The arrow indicates the putative start point and direction of translation and transcription. The location of primer 1 is indicated by an arrow.

albumin as the standard. The results indicated that expression of the *tcmG* promoter was 3,300-fold higher than that of the *tcmG*^{d} promoter in the wild-type strain (865 versus 0.25 mU/mg of protein, respectively) and that the *tcmP* promoter was expressed at a 480-fold-lower level than the *tcmG* promoter (1.8 versus 865 mU/mg of protein, respectively). A qualitative plate assay showed that the *tcmG* and *tcmG*^{d} promoters apparently are also active in *E. coli*; no activity was observed for the *tcmP* promoter nor the vector alone (data not shown).

Concluding remarks. The fact that TCM C biosynthesis takes place in the *S. lividans*(pWHM1026) strain does not exclude the possibility that some enzymatic activities or cofactors are supplied by *S. lividans* (in view of its close similarity to *Streptomyces coelicolor* [7], this organism must have at least three sets of polyketide synthase or fatty acid synthase genes, for instance) and thus by genes lying outside the 12.6-kb *tcm* region in *S. glaucescens*. Oxytetracycline biosynthesis in *Streptomyces rimosus* is dependent on a specific 5-deazaflavin (22), and the genes for the biosynthesis of this cofactor are located outside the antibiotic cluster (29). Acetyl coenzyme A and malonyl coenzyme A transacylase activities have been postulated to be required for the biosynthesis of the tetracenomycin decaketide (2, 33), and both of these activities might be supplied by the primary metabolism of the cell.

We currently have no evidence from gene mutation, sequencing, and expression experiments for a cluster-associated *tcm* regulatory gene. The above result does not necessarily settle this question, because *S. lividans* is likely to contain as part of its *act* cluster an *actII orf4* homolog (14) that could provide the necessary positively acting determinant. Nonetheless, TCM C production by the *S. lividans*(pWHM1026) strain implies that genetic regulation of TCM C biosynthesis could be different from the biosynthesis of spiramycin in *Streptomyces ambofaciens* (14a), actinorhodin in *S. coelicolor* (14), streptomycin in *Streptomyces griseus* (12), bialaphos in *Streptomyces hygroscopicus* (28), and

daunorubicin in *Streptomyces peucetius* (36), in which specific, positively acting regulatory genes are closely linked to the antibiotic biosynthesis and resistance genes. A 1,700-bp region downstream of *tcmO* (Fig. 1) has been sequenced (11), but no mutations affecting TCM C biosynthesis map to this area (23). Moreover, low-resolution S1 mapping experiments within this region have revealed the presence of a convergently transcribed gene, and sequence analysis suggests that it represents the 3′ end of an ATP synthase subunit (11), which we feel is unlikely to serve as a regulator of TCM C production. On the other hand, until the region downstream of *tcmR* (Fig. 1) has been sequenced or deleted, we must consider that it could contain a gene that regulates TCM C production but is dispensable in *S. lividans*.

We propose that the 12 *tcm* genes are expressed as four principal transcripts: three monocistronic ones for the *tcmAR* resistance and repressor genes (15, 16) and *tcmP* (10) and a polycistronic one for the *tcmGHIJKLMNO* genes. That the *tcmGHIJKLMNO* genes could constitute an operon under control of the *tcmG* promoter comes from the knowledge that the *tcmIc* mutant is unable to make any of the intermediates of TCM C biosynthesis (42) and is able only to bioconvert TCM E to TCM A2 (24). The latter conclusion is based on the fact that the *tcmIc* mutant cosynthesizes TCM C only in mixed cultures with the *tcmVII* strain, which contains an inactive *tcmP* gene (10); hence, the *tcmIc* mutant must convert TCM E, excreted by the *tcmVII* mutant, into TCM A2, which then serves as a substrate for the biosynthesis of TCM C by the *tcmVII* mutant. The *tcmIc* mutant also lacks the products of the *tcmKLMN* polyketide synthase genes (18, 33). Furthermore, two polar *tcm* insertion mutations, represented by the *S. glaucescens* WMH1074 and WMH1076 *tcmIb* strains (originally GLA.2-40 and 16-15, respectively [24]), do not produce any of the intermediates of TCM C biosynthesis and cosynthesize TCM C only with the *tcmIII* (*tcmG* mutant) and *tcmVII* (*tcmP* mutant) strains (10, 24). These insertions map near the *Bgl*II site in the *tcmJ* gene and, by Southern analysis, contain 1.1 and 0.2 kb of extra DNA, respectively (Fig. 1) (11). The unique cosynthesis behavior of these mutants indicates that the *tcmIb* mutations abolish the function of the *tcmKLMNO* genes, which argues against the presence of additional promoters downstream of *tcmJ* and supports the idea of a single operon for the *tcmGHIJKLMNO* genes. Finally, in preliminary S1 nuclease protection experiments, we have observed apparent full-length protection of a set of overlapping single-strand DNA probes designed to test whether the *tcmGHIJKLMNO* genes are transcribed as a single polycistronic mRNA species (11).

Interestingly, the apparent transcriptional and putative translational start points of *tcmP* are identical (Fig. 4B). Initiation of translation and transcription at the same site is an unusual feature in procaryotes (for a review, see reference 35), and 8 of 11 known examples are involved in the secondary metabolism of *Streptomyces* spp. (antibiotic resistance and differentiation) (35). In 10 of the 11 examples, the presumed translation start codon is AUG (35), a further coincidence with the *tcmP* gene. These mRNA species lack a conventional ribosome binding site, and this might be important for the regulation of translation of *tcmP*, perhaps along with the noted presence of rare codons in this message (10).

The *tcmG* promoter (Fig. 4A), characterized by the results of S1 mapping and primer extension experiments plus the similarity of the −10 and −35 regions to other promoter sequences in the *Streptomyces* genus (35), displays an apparent similarity of the −35 region to the consensus recognition sequence for RNA polymerases containing σ^{70}-like factors (17), whereas only the first and last nucleotides of its −10 region represent conserved nucleotides in *Streptomyces* promoters (35). The fact that a T-to-G transversion in the −10 hexamer of this promoter decreased its activity drastically in *S. glaucescens* supports this assignment. Even though we favor the foregoing interpretation, an alternative assignment of the −10 region for the *tcmG* promoter could be accomplished by placing the T that was substituted by a G in the *tcmIc* mutant at a different position of the −10 hexamer (Fig. 4A, *tcmG** promoter), since it is well known that this position in the −10 hexamer of σ^{70}-like promoter sequences is a highly conserved T (17). Mutagenesis of the latter position in at least one actinomycete promoter resulted in reduction of the transcript level below levels detectable by S1 nuclease protection experiments (3, 4). The corresponding −35 region of the *tcmG** promoter would have negligible similarity to the −35 hexamer of σ^{70}-like promoter sequences (Fig. 4A), yet this lack of similarity in the −35 region to σ^{70}-like promoter sequences has been observed for many *Streptomyces* promoters of genes involved in secondary metabolism (35).

Work can now be done to study more thoroughly the activity of the *tcmG* and *tcmP* promoters as a function of growth stage and nutrient sources and to examine whether the region between the *tcmG* and *tcmO* genes contains additional promoters that are active under certain physiological conditions.

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REFERENCES

1. **Aldea, M., F. Claverie-Martin, M. R. Diaz-Torres, and S. R. Kushner.** 1988. Transcript mapping using [^{35}S]-DNA probes, trichloracetate solvent and dideoxy sequencing ladders: a rapid method for identification of transcriptional start points. Gene **65:**101–110.
2. **Bibb, M. J., S. Biro, H. Motamedi, J. F. Collins, and C. R. Hutchinson.** 1989. Analysis of the nucleotide sequence of the *Streptomyces glaucescens tcmI* genes provides key information about the enzymology of polyketide antibiotic biosynthesis. EMBO J. **8:**2727–2736.
3. **Bibb, M. J., and G. R. Janssen.** 1987. Unusual features of transcription and translation of antibiotic resistance genes in antibiotic-producing *Streptomyces*, p. 309–318. *In* M. Alacevic, D. Hranueli, and Z. Toman (ed.), Genetics of industrial microorganisms: proceedings of the Fifth International Symposium. Pliva, Zagreb, Yugoslavia.
4. **Bibb, M. J., G. R. Janssen, and J. M. Ward.** 1985. Cloning and analysis of the promoter region of the erythromycin-resistance gene (*ermE*) of *Streptomyces erythraeus*. Gene **41:**E357–E368.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Brosius, J., R. L. Cate, and A. P. Perlmutter.** 1982. Precise location of two promoters for the β-lactamase gene of pBR322. J. Biol. Chem. **257:**9205–9210.
7. **Chater, K. F., and D. A. Hopwood.** 1989. Antibiotic biosynthesis in *Streptomyces*, p. 129–150. *In* D. A. Hopwood and K. F. Chater (ed.), Genetics of bacterial diversity. Academic Press Ltd., London.

8. **Chomczynski, P., and N. Sacchi.** 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
9. **Clayton, T. M., and M. J. Bibb.** Unpublished data.
10. **Decker, H., H. Motamedi, and C. R. Hutchinson.** 1993. The nucleotide sequences and heterologous expression of *tcmG* and *tcmP*, biosynthetic genes for tetracenomycin C in *Streptomyces glaucescens*. J. Bacteriol. **175:**3876–3886.
11. **Decker, H., R. G. Summers, E. Wendt-Pienkowski, and C. R. Hutchinson.** Unpublished data.
12. **Distler, J., K. Mansouri, G. Mayer, M. Stockmann, and W. Piepersberg.** 1992. Streptomycin biosynthesis and its regulation in streptomycetes. Gene **115:**105–111.
13. **Eckstein, F.** 1985. Nucleoside phosphorothioates. Annu. Rev. Biochem. **54:**367–402.
14. **Fernandez-Moreno, M. A., J. L. Caballero, D. A. Hopwood, and F. Malpartida.** 1991. The *act* cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *Streptomyces*. Cell **66:**769–780.

14a. **Geistlich, M., R. Losick, J. R. Turner, and R. N. Rao.** 1992. Characterization of a novel regulatory gene governing the expression of a polyketide synthase gene in *Streptomyces ambofaciens*. Mol. Microbiol. **6:**2019–2029.

15. **Guilfoile, P. G., and C. R. Hutchinson.** 1992. Sequence and transcriptional analysis of the *Streptomyces glaucescens tcmAR* tetracenomycin C resistance and repressor gene loci. J. Bacteriol. **174:**3651–3658.
16. **Guilfoile, P. G., and C. R. Hutchinson.** 1992. The *Streptomyces glaucescens* TcmR protein represses transcription of the divergently oriented *tcmR* and *tcmA* genes by binding to an intergenic operator region. J. Bacteriol. **174:**3659–3666.
17. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of the *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11:**2237–2255.
18. **Hutchinson, C. R., H. Decker, H. Motamedi, B. Shen, R. G. Summers, E. Wendt-Pienkowski, and W. L. Wessel.** Molecular genetics and biochemistry of a bacterial type II polyketide synthase. *In* R. H. Baltz, T. Ingolia, and G. Hegeman (ed.), Genetics and molecular biology of industrial microorganisms, 2nd ed., in press. American Society for Microbiology, Washington, D.C.
19. **Ingram, C., M. Brawner, P. Youngman, and J. Westpheling.** 1989. *xylE* functions as an efficient reporter gene in *Streptomyces* spp.: use for the study of *galP1*, a catabolite-controlled promoter. J. Bacteriol. **171:**6617–6624.
20. **Leskiw, B. K., M. J. Bibb, and K. F. Chater.** 1991. The use of a rare codon specifically during development? Mol. Microbiol. **5:**2861–2867.
21. **Leskiw, B. K., E. J. Lawlor, J. M. Fernandez-Abalos, and K. F. Chater.** 1991. TTA codons in some genes prevent their expression in a class of developmental, antibiotic-negative, *Streptomyces* mutants. Proc. Natl. Acad. Sci. USA **88:**2461–2465.
22. **McCormick, J. R. D., and G. O. Morton.** 1982. Identity of cosynthetic factor 1 of *Streptomyces aureofaciens* and fragment FO from coenzyme F420 of *Methanobacterium* species. J. Am. Chem. Soc. **104:**4014–4015.
23. **Motamedi, H., and C. R. Hutchinson.** 1987. Anthracycline antitumor antibiotics: organization of the genes for tetracenomycin C production in *Streptomyces glaucescens*, p. 355–362. *In* M. Alacevic, D. Hranueli, and Z. Toman (ed.), Genetics of industrial microorganisms: proceedings of the Fifth International Symposium. Pliva, Zagreb, Yugoslavia.
24. **Motamedi, H., E. Wendt-Pienkowski, and C. R. Hutchinson.** 1986. Isolation of tetracenomycin C-nonproducing *Streptomyces glaucescens* mutants. J. Bacteriol. **167:**575–580.
25. **Motamedi, H., E. Wendt-Pienkowski, R. L. Rubin, R. G. Summers, and C. R. Hutchinson.** Unpublished data.
26. **Neal, R. J., and K. F. Chater.** 1991. Bidirectional promoter and terminator regions bracket *mmr*, a resistance gene embedded in the *Streptomyces coelicolor* A3(2) gene cluster encoding methylenomycin production. Gene **100:**75–83.
27. **Pulido, D., and A. Jimenez.** 1987. Optimization of gene expression in *Streptomyces lividans* by a transcription terminator. Nucleic Acids Res. **15:**4227–4240.
28. **Raibaud, A., M. Zalacain, T. G. Holt, R. Tizard, and C. J. Thompson.** Nucleotide sequence analysis reveals linked *N*-acetyl hydrolase, thioesterase, transport, and regulatory genes encoded by the bialaphos biosynthetic gene cluster of *Streptomyces hygroscopicus*. J. Bacteriol. **173:**4454–4463.
29. **Rhodes, P. M., N. Winskill, E. J. Friend, and M. Warren.** 1981. Biochemical and genetic characterization of *Streptomyces rimosus* mutants impaired in oxytetracycline biosynthesis. J. Gen. Microbiol. **124:**329–338.
30. **Sala-Trepat, J. M., and W. C. Evans.** 1971. The meta cleavage of catechol by *Azotobacter* species: 4-oxalocrotonate pathway. Eur. J. Biochem. **20:**400–413.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Sharrocks, A. D., and D. P. Hornby.** 1991. S1 nuclease transcript mapping using Sequenase-derived single stranded probes. BioTechniques **10:**426–428.
33. **Shen, B., R. G. Summers, H. Gramajo, M. J. Bibb, and C. R. Hutchinson.** 1992. Purification and characterization of the acyl carrier protein of the *Streptomyces glaucescens* tetracenomycin C polyketide synthase. J. Bacteriol. **174:**3818–3821.
34. **Stein, D. S., K. J. Kendall, and S. N. Cohen.** 1989. Identification and analysis of transcriptional regulatory signals for the *kil* and *kor* loci of *Streptomyces* plasmid pIJ101. J. Bacteriol. **171:**5768–5775.
35. **Strohl, W. R.** 1992. Compilation and analysis of DNA sequences associated with apparent *Streptomyces* promoters. Nucleic Acids Res. **20:**961–974.
36. **Stutzman-Engwall, K. J., S. L. Otten, and C. R. Hutchinson.** 1992. Regulation of secondary metabolism in *Streptomyces* spp. and overproduction of daunorubicin in *Streptomyces peucetius*. J. Bacteriol. **174:**144–154.
37. **Summers, R. G., E. Wendt-Pienkowski, H. Motamedi, and C. R. Hutchinson.** 1992. Nucleotide sequence of the *tcmII-tcmIV* region of the tetracenomycin C biosynthetic gene cluster of *Streptomyces glaucescens* and evidence that the *tcmN* gene encodes a multifunctional cyclase–dehydratase–*O*-methyl transferase. J. Bacteriol. **174:**1810–1820.
38. **Tsai, J. F. Y., and C. W. Chen.** 1987. Isolation and characterization of *Streptomyces lividans* mutants deficient in intraplasmid recombination. Mol. Gen. Genet. **208:**211–218.
39. **Vara, J., M. Lewandowska-Skarbek, Y.-G. Wang, S. Donadio, and C. R. Hutchinson.** 1989. Cloning of genes governing the deoxysugar portion of the erythromycin biosynthesis pathway in *Saccharopolyspora erythraea* (*Streptomyces erythreus*). J. Bacteriol. **171:**5872–5881.
40. **Weber, W., H. Zähner, J. Siebers, K. Schröder, and A. Zeeck.** 1979. Stoffwechselprodukte von Mikroorganismen. 175. Mitteilung. Tetracenomycin C. Arch. Microbiol. **121:**111–116.
41. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
42. **Yue, S., H. Motamedi, E. Wendt-Pienkowski, and C. R. Hutchinson.** 1986. Anthracycline metabolites of tetracenomycin C-nonproducing *Streptomyces glaucescens* mutants. J. Bacteriol. **167:**581–586.
43. **Zukowski, M. M., D. F. Gaffney, D. Speck, M. Kauffmann, A. Findeli, A. Wisecup, and J. P. Lecoq.** 1983. Chromogenic identification of genetic regulatory signals in *Bacillus subtilis* based on expression of a cloned *Pseudomonas* gene. Proc. Natl. Acad. Sci. USA **80:**1101–1105.