The RNA Polymerase Omega Factor RpoZ Is Regulated by PhoP and Has an Important Role in Antibiotic Biosynthesis and Morphological Differentiation in *Streptomyces coelicolor*†

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In bacteria, the RNA polymerase (RNAP) complex plays a central role in transcription and is a target for regulation of primary metabolism (6, 7, 44). The *rpoZ* gene encodes the RNAP omega (ω) subunit, which forms a complex with the α2β′ core of this enzyme. The ω subunit has been identified in the RNAPs of most free-living bacteria. This protein is functionally homologous to the RpoK subunit of the archaeal RNA polymerase complex and the RNA polymerase subunit of the eukaryotic RNA polymerases I, II, and III (32). In *Escherichia coli* the ω subunit interacts with the β′ subunit and promotes assembly of the RNA polymerase complex (14, 32), although it is not essential for survival in this bacterium (13).

*Streptomyces* spp. are soil-dwelling bacteria that are notorious for their ability to produce thousands of antibiotics, pigments, antitumor agents, immunomodulators, and a variety of other bioactive secondary metabolites (1, 2, 8). Differential expression of secondary metabolism genes occurs following nutrient depletion (34), but the transcriptional control mechanisms that govern the onset of secondary metabolites are still obscure (29). The *rpoZ* gene of *Streptomyces kasugaensis* has been shown to be required for antibiotic production and morphological differentiation but is not essential for growth (21). A DNA fragment containing the *rpoZ* gene was shown to complement an *S. kasugaensis* pleiotropic mutant deficient in aerial mycelium formation and kasugamycin biosynthesis. Although sigma factors in *Streptomyces* have received considerable attention in relation to the expression of antibiotic biosynthetic genes (9, 19, 20), the role of the RNAP ω subunit is still obscure.

The expression of many genes involved in antibiotic biosynthesis is negatively controlled by the phosphate concentration in the medium (reviewed in references 28 and 30). Limitation of inorganic phosphate produces drastic changes in primary metabolism and triggers the onset of secondary metabolism (34).

Phosphate control over the expression of several genes involved in primary or secondary metabolism (37) is mediated by the two-component system PhoR-PhoP (45, 46). Binding of the response regulator PhoP to specific sequences (named PHO boxes) in the promoter regions of phosphate-controlled genes allowed us to identify the consensus binding sequence of the PhoP operator in *Streptomyces coelicolor* (47).

Bioinformatic studies revealed that the *rpoZ* gene has a highly conserved PHO box in its promoter region. Taking into account the putative role of RpoZ in the transcription of genes involved in differentiation and secondary metabolism biosynthesis and its possible regulation by PhoP, it was of utmost interest to study the role of *rpoZ* in antibiotic biosynthesis and morphological differentiation in *S. coelicolor* and its possible regulation by PhoP. In this work, we report the deletion of *rpoZ* via the REDIRECT technique (16), phenotype restoration when a copy of the *rpoZ* wild-type allele is introduced, and *in vivo* and *in vitro* studies on the effect of PhoP on the expression of the *rpoZ* gene. The results indicate an important role of the RpoZ protein in sporulation and antibiotic biosynthesis. The work also establishes *rpoZ* as a new member of the *pho* regulon in *S. coelicolor*.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Streptomyces coelicolor strains M145 (18) and INB201 (ApHpoP) (40) and gmk/TS15062 and rpoZ mutant strains (isolation) were cultivated according to standard procedures (18). TBO medium (17) was used to obtain spores. S. coelicolor liquid cultures were grown in defined MG-3.2 medium containing starch (Scharlau) (50 g liter$^{-1}$), glutamate (8.83 g liter$^{-1}$), and phosphate (3.2 mM) (39). One hundred milliliters of MG-3.2 medium in 500-ml baffled flasks was inoculated with 10$^6$ spores ml$^{-1}$ and incubated at 30°C and 300 rpm for dispersed and reproducible growth. Samples for antibiotic production and growth were taken after 35, 47, 52, 70, 80, and 100 h of growth, and samples for promoter activity determination were taken after 41, 44, 47, 49, and 65 h of growth. For the solid cultures TBO, MS, ISP4, TSA, R5, and YPD media (18) were inoculated with a suspension of 10$^6$ spores ml$^{-1}$ and grown at 30°C (normal conditions) or 40°C (for temperature resistance assay).

rpoZ disruption by gene replacement. The rpoZ deletion mutant was obtained by REDIRECT technology (16). Two primers (rpoZ-F $5'$-CCAGCTCTGATCAGGACGCT-3') and rpoZ-R $5'$-GCGAGGTGCAAGGTTATTTCGCCTCCGAGCCCCACC-3') were designed for the amplification of the rpoZ region on pFh174. The PCR product was electroporated in E. coli BW25113/pIJ790 carrying the cosmid St9CS, an S. coelicolor cosmid containing target SCO1478. Since E. coli BW25113/pIJ790 has a high recombinant activity, replacement of the rpoZ coding region by the rpoZ resistance cassette taken from high efficiency transformation vector B. subtilis was performed. The recombinant strains were transformed in E. coli ET12567/pLZ2802 and then transferred to Streptomyces by conjugation. Deletion mutants were selected by their Apr$^+$ phenotype and confirmed by PCR and Southern hybridization.

Complementation of $\Delta rpoZ$ strains. A 550-bp fragment of S. coelicolor M145 that contains the rpoZ gene with its promoter and terminator sequences was amplified with primers RPOZ-Cut $5'$-TCTGTGCTGATGCTGCTGGTAC-3' and RPOZ-3' $5'$-AGGCGCTCTTAAATCTCATCGCA-3', which had been modified with XbaI and BamHI restriction sites, respectively. The PCR product was cloned into pRA by REDIRECT technology (16). Two primers (rpoZ-F $5'$-CCAGCTCTGATCAGGACGCT-3') and rpoZ-R $5'$-GCGAGGTGCAAGGTTATTTCGCCTCCGAGCCCCACC-3') were designed for the amplification of the rpoZ region on pFh174. The PCR product was electroporated in E. coli BW25113/pIJ790 carrying the cosmid St9CS, an S. coelicolor cosmid containing target SCO1478. Since E. coli BW25113/pIJ790 has a high recombinant activity, replacement of the rpoZ coding region by the rpoZ resistance cassette taken from high efficiency transformation vector B. subtilis was performed. The recombinant strains were transformed in E. coli ET12567/pLZ2802 and then transferred to Streptomyces by conjugation. Deletion mutants were selected by their Apr$^+$ phenotype and confirmed by PCR and Southern hybridization.

Disruption of the gmk gene. The SCO1479 (gmk-) disrupted mutants were constructed using the apramycin-resistant $\Delta rpoZ$ mutant strain, a 1.4-kb fragment containing the Tn5 insert. The internal fragment of the Aprr gene (0.9 kb) was amplified by PCR using total DNA as the template and the primers 5'-TTCCCATCCATCGGAAGGTAGAGCGTGATTCCGGGATCCGTCGACC-3' and 5'-AGGCGCTCTTAAATCTCATCGCA-3'. The amplified fragment was digested with XbaI and BamHI restriction sites, respectively. The PCR product was cloned into the BamHI and NdeI cloning sites with respect to the translation start triplet of SCO1478 and the last 176 nucleotides (nt) of the SCO1479 coding region. The BamHI and NdeI cloning sites were modified with XbaI and BamHI restriction sites, respectively. The PCR product was cloned into pRA by REDIRECT technology (16). Two primers (rpoZ-F $5'$-CCAGCTCTGATCAGGACGCT-3') and rpoZ-R $5'$-GCGAGGTGCAAGGTTATTTCGCCTCCGAGCCCCACC-3') were designed for the amplification of the rpoZ region on pFh174. The PCR product was electroporated in E. coli BW25113/pIJ790 carrying the cosmid St9CS, an S. coelicolor cosmid containing target SCO1478. Since E. coli BW25113/pIJ790 has a high recombinant activity, replacement of the rpoZ coding region by the rpoZ resistance cassette taken from high efficiency transformation vector B. subtilis was performed. The recombinant strains were transformed in E. coli ET12567/pLZ2802 and then transferred to Streptomyces by conjugation. Deletion mutants were selected by their Apr$^+$ phenotype and confirmed by PCR and Southern hybridization.

RESULTS

The SCO1478 open reading frame (ORF) encodes a small protein homologous to RpoZ. The SCO1478 gene of S. coelicolor encodes a small protein (90 amino acids) with a predicted mass of 9.7 kDa that is homologous to the RpoZ of S. kasugaeensis (98% identity) (21) and to a hypothetical protein identified in the genomes of Streptomyces griseus and other Streptomyces species, but it is not similar to the WhiG-like sigma factor designated RpoZ in Streptomyces aureofaciens (22, 23) (see Discussion). The RpoZ protein contains a PRK02950 motif, which is characteristic of the DNA binding domain of the RNA polymerase $\sigma$ subunit.

In E. coli the rpoZ gene is linked to the spoT gene (12), encoding a pyrophosphatase that controls the ppGpp levels. As shown in Fig. 1, the S. coelicolor rpoZ gene is located downstream of a guanylate kinase gene (gmk) that is involved in the biosynthesis of guanine nucleotides (35). Since the gmk-rpoZ arrangement is kept in E. coli and in S. coelicolor (Fig. 1), the possible role of gmk in differentiation and secondary metabolite production was also investigated by gene disruption (see below).

Deletion of the rpoZ gene alters the phenotype of the mutant. In order to elucidate the role of rpoZ, the gene was deleted using the REDIRECT technique. For this purpose, cosmID St95C, containing the pIJ774 cassette replacing rpoZ, was transformed into E. coli ET12567/pUZ8002 (see Materials and Methods). After conjugation into S. coelicolor, fourApr$^+$ Kan$^+$ transformants (named T107, T150, T162, and T165) were selected. The deletion of the rpoZ gene in these four transformants was confirmed by PCR and Southern hybridization. The four $\Delta rpoZ$ transformants behaved similarly, and one of them, T165, was selected for further studies. Complementation of the $\Delta rpoZ$ mutant (T165) was performed by conjugation with E. coli ET12567/pUZ8002 carrying the construction pRA-rpoZ.
and Southern hybridization. The complementation was also confirmed by PCR from its own promoter and carries its own terminator sequence. The complementation was also confirmed by PCR and Southern hybridization.

To determine the phenotype of the $\Delta rpoZ$ mutant, the parental M145, $\Delta rpoZ$ (T165), and complemented (C1) strains were grown on several media. After 2 days of growth, the onset of actinorhodin (ACT) production was clearly precocious in the $\Delta rpoZ$ strain on MS medium (Fig. 2A). On the other hand, S. coelicolor $\Delta rpoZ$ showed delayed growth on all media tested, especially TBO and ISP4 (Fig. 2B), at this time. After 4 days of growth, actinorhodin production was already observed in both the wild-type and complemented strains on R5, TSA, and ISP4 but not on YPD, MS, and TBO. On the other hand, the $\Delta rpoZ$ strain produced significant amounts of actinorhodin on R5, ISP4, MS, and TBO, although not on TSA (Fig. 2C). Sporulation of the $\Delta rpoZ$ mutant was delayed in comparison to those of the parental and complemented strains (Fig. 2B and C). After 6 days, all strains were able to sporulate on TBO, MS, and ISP4, although the spores of the $\Delta rpoZ$ mutant remained white (Fig. 2D). This white phenotype of the mutant remained after 2 weeks of growth. Another interesting observation was the sensitivity of S. coelicolor $\Delta rpoZ$ to high temperatures; indeed, when the temperature was increased to 40°C, the mutant was impaired in aerial mycelium formation, showing a bald phenotype, while the wild type was still able to sporulate (Fig. 2E). However, despite the lack of spore pigmentation and the lower resistance to heat stress, the mutant spores were viable and showed normal morphology, as confirmed by electron microscopy (Fig. 3).

To test if the putative guanylate kinase gene ($gmk$) had any role in differentiation or antibiotic production, this gene was disrupted as described in Materials and Methods. Disruption of $gmk$ did not alter the phenotype of the mutant (data not shown), excluding a role of this gene in the control of growth, differentiation, or secondary metabolism.

In summary, on most of the solid media tested, deletion of $rpoZ$ produced a retardation of growth, a lack of the gray pigmentation, and an early onset of actinorhodin biosynthesis. (see Materials and Methods), in which the $rpoZ$ gene is expressed from its own promoter and carries its own terminator sequence. The complementation was also confirmed by PCR and Southern hybridization.

**ACT and RED pigment production is drastically altered in $\Delta rpoZ$ liquid cultures.** In order to quantify the effect of the $rpoZ$ deletion on growth and antibiotic production in submerged cultures, the $\Delta rpoZ$ mutant, M145, and the complemented $rpoZ$ (C1 and C2) strains were grown in liquid MG-3.2 medium (38, 39, 40, 41, 42). Phosphate is depleted in this medium (the residual level is below 0.1 mM) after 44 h of growth, time in which the $pho$ regulon genes are induced and the onset of secondary metabolism takes place (39, 40).

As shown in Fig. 4, growth of the $\Delta rpoZ$ mutant was slower than that of the parental and complemented strains during the first 70 h of culture. In fact, the phosphate in the medium was depleted in the mutant cultures 10 h later than in the control cultures (indicated by vertical lines in Fig. 4A). However, the final biomass was higher in the mutant strain than in the parental and complemented strains (Fig. 4A). When the cultures were observed under the optical microscope, differences were observed between the wild-type and mutant strains in terms of the shape and complexity of the mycelial pellets. While the parental strain developed branches of mycelium around the initial pellets, which grow throughout the time culture, no branch formation was observed in the $\Delta rpoZ$ mutant cultures (see Fig. S1 in the supplemental material). This observation points toward a role of RpoZ in the control of mycelium development in S. coelicolor in both liquid and solid cultures (26).

In relation to the antibiotic synthesis in liquid cultures, there was a burst of actinorhodin (ACT) production in the $\Delta rpoZ$ mutant at the first stages of culture growth, in contrast to the case for the parental strain, in which antibiotic production took place after 47 h (Fig. 4B). The volumetric ACT values for the parental strain increased from 1.7 ± 0.6 µg ml$^{-1}$ at 47 h to 254 ± 27 µg ml$^{-1}$ at 70 h, in contrast to the case for the $\Delta rpoZ$ mutant, where the volumetric ACT values did not change from 47 h (28.7 ± 0.3 µg ml$^{-1}$) to 70 h (29.4 ± 1 µg ml$^{-1}$). This indicates that the synthesis of ACT is precocious in the mutant strain but that it fails to maintain the normal production rate after 47 h. This phenomenon agrees with the clear burst of ACT production observed in complex MS and TBO solid media, where the $\Delta rpoZ$ mutant strain started to produce ACT sooner than the parental strain, in spite of the significant delay in growth of the mutant (Fig. 2A and C). In conclusion, the onset of actinorhodin biosynthesis took place earlier in the $\Delta rpoZ$ mutant, although maximum ACT values were quite low in comparison to those in the parental M145 strain.

A more drastic effect of the $rpoZ$ deletion on undecylprodigiosin (RED) production was observed. Almost no detectable synthesis of the RED pigment was observed in the $\Delta rpoZ$ strain, at least until very late time points, when a low production of this pigment took place (Fig. 4C). Strikingly, while in the parental strain ACT production and RED production took

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** Physical map of the S. coelicolor DNA region containing the $rpoZ$ gene (SCO1478). Note the linkage to the $gmk$ gene (SCO1479), encoding a putative guanylate kinase. For comparison, the E. coli DNA region containing the $rpoZ$, $gmk$, and spoT genes is shown at the bottom.
place almost simultaneously, with RED production closely followed by ACT production, in the mutant the synthesis of these antibiotics occurred in very separate stages of the culture (with the ACT pigment produced first and the RED one later).

Almost full restoration of ACT production and partial restoration of RED production were observed in the complemented strain (Fig. 4B and C). This lack of full restoration is due to the effect of integration of the plasmid on ACT and RED production, as was shown when the plasmid without the complementation cassette was introduced in the wild-type strain (Fig. 4B and C). This indicates that the drastic reduction in formation of these secondary metabolites is due to the absence of a functional RpoZ (see Discussion). Not all secondary metabolites are regulated in the same manner. The production of calcium-dependent antibiotic (CDA) in nutrient agar was slightly increased in the \( \Delta \text{rpoZ} \) mutant strain compared to the parental strain, and its production was restored to the parental levels in the complemented strain (Fig. 5).

Binding of PhoP to the promoter region of rpoZ. Inorganic phosphate is known to control expression of genes involved in both primary and secondary metabolism. Many of the primary metabolism genes are controlled by direct binding of PhoP to their promoter regions (37, 47), whereas secondary metabolism genes are regulated through signal transduction cascades involving pathway-specific regulators (29, 40).

Bioinformatic studies indicated that the region upstream of rpoZ contains two direct repeat units, designated DRu-1 and DRu-2, i.e., a PHO box. They are adjacent and located at 33 nt from the rpoZ translation start triplet. The individual information content (the \( R_i \) value) (43) of each 11-nt DRu obtained using model I of the PhoP binding site (47) indicates that the two DRus have a high score (9.7 and 7.3 bits for DRu-1 and DRu-2, respectively), although these values were lower than in the consensus PHO box (\( R_i \) 14.6 bits) since the second nucleotides of both DRu-1 and DRu-2 did not match the consensus.
T nucleotide. This feature is also observed in the promoter region of one of the major sigma factor-encoding genes (hrdA) in S. coelicolor, which has been previously shown to be bound and repressed by PhoP (37, 47) (Fig. 6A). Therefore, binding of PhoP to the 223-nt DNA fragment carrying the rpoZ promoter region was tested. As shown in Fig. 6B, binding of PhoP

FIG. 4. Growth and production of actinorhodin (ACT) and undecylprodigiosin (RED) by the parental strain M145, the ΔrpoZ mutant, the complemented mutant strains (C1 and C2), and strain M145 plus the plasmid without the complementation cassette (control) in MG-3.2 medium. (A) Dry weight; (B) ACT production; (C) RED production. Black circles, strain M145; black squares, M145 control; white circles, ΔrpoZ mutant; black triangles, complemented strains. The vertical lines indicate when phosphate is depleted in the medium (ΔH1102150 ΔH9262M) in both parental (dashed lines) and ΔH9004rpoZ (dotted lines) cultures. Error bars correspond to the standard errors of the means for three biological replicates in M145 and ΔH9004rpoZ cultures and four replicates for the complemented strains (two cultures of each complemented strain).

FIG. 5. Effect of rpoZ deletion on CDA production. For the bioassay, strain M145, the ΔrpoZ mutant, and C1 (ΔrpoZ mutant complemented with the wild-type rpoZ) were grown on nutrient agar for 48 h. Agar plugs (5 mm in diameter) were then transferred to soft nutrient agar inoculated with Bacillus mycoides. The plate on the left was supplemented with 17 mM calcium nitrate to induce CDA activity. The plate on the right, without calcium nitrate, acts as a control.

FIG. 6. (A) Individual information analysis of the PhoP binding sites in the rpoZ and hrdA promoter regions using the sequence walker method (43) and the model I weight matrix of Sola-Landa et al. (47). Boxes contain the individual information content (Ri, bits) of each 11-nt direct repeat unit (DRu). The height of the letters represents the Ri contribution of each position to the total information content. Letters extending downward represent unfavorable protein-DNA contacts. Note that in both cases the second base is unfavorable for each of the DRus. (B) Electrophoretic mobility shift assays of the rpoZ promoter with the glutathione S-transferase (GST)-PhoPDBD protein and competition with unlabeled probe. Lanes 1 to 5, labeled probe with protein at 2 μM (lane 1) and 4 μM (lane 2), 4 μM protein and a 500× excess of unlabeled unspecific probe (lane 3), 4 μM protein and a 100× excess of unlabeled specific probe (lane 4), and 4 μM protein and a 500× excess of unlabeled specific probe (lane 5).
to this region was clearly observed, giving rise to one shifted band (DNA-PhoP complex), as expected for a core-core (CC)-type operator (47). This result suggests that expression of rpoZ is modulated by PhoP.

PhoP control of rpoZ expression. To check the in vivo effect of the PhoP binding on rpoZ expression, the promoter of this gene was coupled to the luxAB reporter genes in S. coelicolor M145 (black circles) and INB201 (ΔphoP; white squares) strains in MG-3.2 medium. Luciferase specific activity (black lines, left y axis) and growth (gray lines, right y axis) are shown. The time when the phosphate in the medium is depleted (<50 μM) in both M145 and INB201 cultures is represented with a black vertical line. Error bars correspond to the standard errors of the means for four biological replicates (two replicates of two different exconjugants per condition).

FIG. 7. Expression of the rpoZ promoter coupled to the luxAB reporter genes in S. coelicolor M145 (black circles) and INB201 (ΔphoP; white squares) strains in MG-3.2 medium. Luciferase specific activity (black lines, left y axis) and growth (gray lines, right y axis) are shown. The time when the phosphate in the medium is depleted (<50 μM) in both M145 and INB201 cultures is represented with a black vertical line. Error bars correspond to the standard errors of the means for four biological replicates (two replicates of two different exconjugants per condition).

Characterization of the rpoZ promoter. In order to elucidate the molecular basis of the negative role of PhoP in rpoZ expression, the transcription start point (TSP) of rpoZ was determined by primer extension analysis. The aim of the experiment was to determine the position of the PHO box with regard to the TSP and to check whether the TSP is the same in the wild-type and ΔphoP strains. For this purpose, RNA samples from 48-h MG-3.2 phosphate-limited cultures were used. As shown in Fig. 8, the same TSP was obtained in both the wild-type and ΔphoP strains, and the PHO box was located at positions −54 to −33.

As shown in Fig. 8, the TSP of rpoZ coincides with the first nucleotide (G) of its translation start triplet. Although the majority of archaeal transcripts are leaderless, this is not the case with bacterial transcripts (3). In any case, at least 11 leaderless transcripts of different Streptomyces species are described in the review by Strohl (48). Of these 11 transcripts, only one has a GUG as the translation start triplet, so this feature does not seem to be usual in Streptomyces. In all com-
piled promoters, the −10 box ends with a T (48). Taking into account this feature and also the presence of an A (86%) in the second position of the hexamer and the presence of a purine nucleotide in the fourth and fifth positions (69% and 72%, respectively), we have identified the hypothetical −10 element of the rpoZ promoter (Fig. 8). However, no obvious −35 hexamer was found around the −35 region of the rpoZ promoter, and the PHO box was located upstream of this region. In other pho genes, the PHO box is located in the −35 region. PhoP usually acts as a transcriptional activator when it binds to the −35 region. In our case, PhoP acts a transcriptional repressor. This might occur by a change in the conformation of the DNA that affects the transcription process (see Discussion).

**DISCUSSION**

All bacterial RNA polymerases consist of five subunits, α2 (two identical monomers), β, β′, and ω, which perform DNA-dependent transcription (14). In addition, a variety of sigma (σ) factors are used in the recognition of different promoters (9, 19, 20, 27). The role of the omega subunit of the bacterial RNAP complex has remained obscure for decades. In vitro studies with *E. coli* revealed that the ω subunit is an important component of RNAP complex, being required to restore functionality to denatured RNAP (33). In fact, the ω subunit has been shown to interact with the αββ′ core through binding to the β′ subunit (15). This interaction was also defined when the crystal structure of the *Thermus aquaticus* RNAP was determined (32). The presence of the ω subunit is not strictly required for growth, although *E. coli* mutants lacking the ω subunit show a low growth rate and a lower final cell density (50). On the other hand, a truncated version of RpoZ containing the N-terminal region (51 out of 91 amino acids) is sufficient to interact with the αββ′ core, leading to a full growth rate of the complemented strain (15).

The deletion of the rpoZ gene in *Mycobacterium smegmatis* (31) produced a fragmentation of the β′ subunit, although the fragments remained associated with the RNAP core. This mutant showed a reduced growth rate and abnormal colony morphology and pigmentation (31).

The *S. coelicolor* 90-amino-acid RpoZ protein is highly conserved in all sequenced *Streptomyces* genomes. No other rpoZ-like gene was found in the genome of *S. coelicolor*. Surprisingly, an unrelated gene encoding a 278-amino-acid sigma factor homologous to WhiG, which also influences sporulation, was named rpoZ in *Streptomyces averofaciens* (22, 23). However, the homology of *S. coelicolor* RpoZ to the ω subunits of *E. coli* (12), *M. smegmatis* (31), *S. kasugaensis* (21), and other bacteria clearly indicates that the 90-amino-acid protein encoded by SCO1478 is the authentic ω subunit of this actinomycete.

As shown in this article, deletion of the rpoZ gene in *S. coelicolor* causes a reduction of the growth rate, as occurs in *E. coli* and *M. smegmatis*. Disruption of the guanylate kinase gene (gmk), located upstream of rpoZ, had no effect on growth or differentiation. Therefore, gmk and rpoZ appear to be transcribed independently from each other. The slow-growth phenotype of *S. coelicolor* rpoZ mutants in several solid and liquid media suggests that the RNAP complex lacking the ω subunit is inefficient in terms of expression of the genes required for a full growth rate. However, it is clear that RpoZ is not essential for growth, since the rpoZ-defective mutants are able to reach the same levels of growth in prolonged cultures as the parental strain.

The sporulation process was also affected in the ΔrpoZ mutant, although viable spores, with normal morphology, are formed. However, the spores of the mutant lack the typical gray pigment associated with *S. coelicolor* and other *Streptomyces* spores. In addition to the white phenotype, the ΔrpoZ mutant is less resistant to temperature stress than the parental strain. A bald phenotype (no aerial mycelium formation) is obtained when the mutant strain is grown at 40°C. A role of RpoZ in resistance to heat stress in *E. coli* was also reported. In this bacterium, overproduction of RpoZ suppressed the temperature-sensitive phenotype of rpoZ mutants (32).

In this work we show that the deletion of rpoZ in *S. coelicolor* has a very strong effect on antibiotic biosynthesis, particularly on undecylenic acid, almost preventing its expression. Kojima and coworkers (21) observed a similar effect on the biosynthesis of the aminoglycoside kasugamycin and proposed that this might be a general effect, also affecting secondary metabolites other than aminoglycosides. Indeed, in *S. coelicolor*, the deletion of rpoZ affected not only the biosynthesis of the polyclade-derived undecylenic acid but also that of the polyketide actinorhodin, the lipopeptide CDA, and the gray pigment associated with spores, suggesting that RpoZ modulates the expression of different secondary metabolites. These unrelated metabolites were affected differently by the deletion of rpoZ, undecylenic acid and the gray pigment were almost abolished in the ΔrpoZ mutant, whereas CDA synthesis increased. On the other hand, production of actinorhodin was impaired after an initial burst in the first stages of the culture. The precocious production of actinorhodin by the ΔrpoZ mutant may be explained as the result of defective RNAP complex formation which induces a low growth rate that triggers a burst of this antibiotic. These differences are likely to be due to the distinct organization and nucleotide sequences of promoters in the actinorhodin, undecylenic acid, or CDA clusters that affect their interaction with the RNAP complex and the ω subunit.

The molecular mechanism of interaction of the RNAP complex with promoters of genes controlling secondary metabolism is also modulated by the action of specific DNA binding proteins such as PhoP or AfsR (40). AfsR seems to act by recruiting the RNAP complex to interact with higher affinity with those AfsR-regulated promoters (49). AfsR is, indeed, a positive regulator of afsS (encoding a sigma factor-like small protein) that triggers expression of the pathway-specific regulators ActII-ORF4 and RedD (11).

PhoP recognizes a DNA binding sequence similar to that of AfsR (40) and appears to act by a similar mechanism, recruiting the RNAP complex for interaction with the afsS promoter. In *E. coli* and *Bacillus subtilis*, the PhoB protein (homologous to the *Streptomyces* PhoP) also interacts with the RNAP holoenzyme (5, 25). In summary, the RpoZ protein may affect expression of secondary metabolite genes by interaction with the core RNAP and with wide-domain regulators such as AfsR or PhoP.

As shown in this work, PhoP binds to the rpoZ promoter, in agreement with the presence in its promoter of a consensus
PHO box. Two direct repeats, DRu-1 and DRu-2, are adjacent, forming a class I (CC) operator (47). Although both DRus have an unconserved nucleotide in the second position, PhoP was shown to bind the promoter with high affinity. The binding of PhoP to this region formed a single DNA-protein complex, in agreement with the behavior of class I operators. The effect of PhoP on this promoter is negative, since the promoter activity was higher in the ΔphoP mutant than in the parental strain.

According to Browning and Busby (4), there are three general mechanisms of transcriptional repression. In the first mechanism, the repressor binds in or close to the core of the promoter elements and produces a steric hindrance of RNA polymerase binding. In the second, the repressor binds to promoter-distal sites and does not prevent binding of RNA polymerase to the promoter but instead interferes with postrecruitment steps in transcription initiation. In the third mechanism, the repressor functions as an antiactivator.

We have previously described that PhoP acts as a repressor with the first mechanism mentioned above. Thus, PhoP represses pitH2 transcription when it binds overlapping the –10 element (39). PhoP also has been shown to function as an antiactivator (third mechanism). PhoP represses the glaA and afsS genes competing with the binding sites of the transcriptional activators GlnR and AfsR (38, 40, 42). In this study, we also describe PhoP acting as a transcriptional repressor according to the second mechanism. Thus, rpoZ expression is slightly decreased when PhoP binds upstream of the promoter elements. Only the steric hindrance mechanism (where the binding of the RNA polymerase is prevented when PhoP binds to the –10 hexamer) seems to produce a drastic repression effect on the transcription of the genes controlled by this protein.

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