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bldA-Dependent Expression of the Streptomyces exfoliatus M11 Lipase Gene (lipA) Is Mediated by the Product of a Contiguous Gene, lipR, Encoding a Putative Transcriptional Activator

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Extracellular lipase synthesis by Streptomyces lividans 66 carrying the cloned lipase gene (lipA) from Streptomyces exfoliatus M11 was found to be growth phase dependent, since lipase was secreted into the medium mainly during the stationary phase; S1 nuclease protection experiments revealed abundant lipA transcripts in RNA preparations obtained during the stationary phase but not in those obtained during exponential growth. Transcription from the lipA promoter was dependent on the presence of lipR, a contiguous downstream gene with a very high guanine-plus-cytosine content (80.2%). The deduced lipR product consists of a protein of 934 amino acids that shows similarity to known transcriptional activators and has a strong helix-turn-helix motif at its C terminus; this motif is part of a domain homologous to DNA-binding domains of bacterial regulators of the LuxR/LuxB superfamily. The lipR sequence revealed the presence of a leucine residue, encoded by the rare TTA codon, which caused bldA dependence of lipA transcription in Streptomyces coelicolor A3(2); replacement of the TTA codon by the alternate CTC leucine codon alleviated bldA dependence but not the apparent growth phase-dependent regulation of lipA transcription. When lipR expression was induced in a controlled fashion during the exponential growth phase, by placing it under the inducible lipA promoter, lipase synthesis was shifted to the exponential growth phase, indicating that the timing of lipR expression, and not its bldA dependence, is the main cause for stationary-phase transcription of lipA.

Members of the genus Streptomyces are gram-positive soil eubacteria characterized by a mycelial growth habit. Their complex life cycle involves both morphological and physiological differentiation (5); morphological and physiological differentiation process (5); among others, the product of the bldA gene is essential for morphological differentiation, and in addition it is the product of a contiguous gene, 

The cloning of lipase-encoding genes from Streptomyces exfoliatus M11 (formerly Streptomyces sp. strain M11) and Streptomyces albus G and shown that similar genes might be commonly found among Streptomyces species (6, 34). In addition, we have shown that these Streptomyces lipases are similar to one of the three lipases (lipase 1) of the psychrotroph Moraxella sp. strain TA144 (8); the similarity of bovine isofrom II of platelet-activating factor acetylhydrolase to the Streptomyces lipases has also recently been described (14). Previous observations suggested that an accessory gene was necessary to achieve a high level of expression of the cloned S. exfoliatus M11 lipase gene in Streptomyces lividans, since cloning the structural gene alone in the absence of downstream sequences present in the insert of the original clone resulted in a much smaller amount of lipase being secreted into the medium (34). In this paper we report that bldA-dependent expression of the S. exfoliatus M11 lipase gene, lipA, is mediated by the product of a contiguous gene, lipR, which encodes a putative transcriptional activator.

MATERIALS AND METHODS

Bacterial strains, plasmids, and microbiological procedures. The Escherichia coli K-12 strain used throughout this study was JM101 (49), which was grown under standard conditions (29); transformation of E. coli with plasmid DNA was carried out as described by Inoue et al. (17). Wild-type S. lividans 66 was used throughout this study (strain 1326 from the John Innes Institute collection); the Streptomyces coelicolor A3(2) strains used were J1501 (hisA1 unA1 strA1 pgl SCP1· SCP2) and its isogenic bldA39 derivative, J1700 (kindly provided by K. F. Chater). Transformation of Streptomyces strains with plasmid DNA was carried out as described by Hopwood et al. (16) except that protoplasts were plated in a hypertonic soft agar overlay (28). The plasmids used are listed in Table 1.

DNA manipulation and sequence analysis. Subcloning of DNA fragments was done by standard procedures (38). Nucleotide sequencing of both strands of the pBI3 insert was done as described previously (34). Sequence assembly and analysis were carried out with PCGENE release 6.85 from IntelliGenetics; in

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particular, the programs ASSEMBGEL, PCOMPARE, PALIGN, REGULAT, and CLUSTALW were used. Database searches were done with the BLASTP program (1). In order to subclone the lipA and lipR genes under the control of the tipA promoter region present in the inducible expression vector plJ6021 (44), NdeI sites were introduced at their respective translational start codons; to change the TTA codon in the lipR sequence, it was replaced by the alternate leucine codon CTC, thereby generating an SsrI site. These sites were introduced either by oligonucleotide-directed mutagenesis according to the method of Kunkel (23) with a U-DNA mutagenesis kit from Boehringer Mannheim or by PCR with AmpliTaq DNA polymerase from Perkin-Elmer. All mutations were confirmed by DNA sequencing.

Growth of cultures and assay of lipase activity. Luria-Bertani medium (29) supplemented with 34% sucrose and 50 mm of antifoam A (Sigma) was used. Cultures (30 ml) were grown at 37°C in 250-ml siliconized flasks with stainless steel stirrups (16) at 250 rpm in an orbital shaker. Thiostrepton was added at 20 μg ml⁻¹ for plasmid maintenance in liquid cultures and at 5 μg ml⁻¹ to induce the tipA promoter; hygromycin B was added at 50 μg ml⁻¹ and kanamycin was added at 100 μg ml⁻¹. Cultures were inoculated with spores washed twice in sterile distilled water, except for the bldA39 parent, J1501, which were inoculated with small mycelial fragments pregrown in Luria-Bertani medium (29)

<table>
<thead>
<tr>
<th>Plasmid</th>
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<tr>
<td>pB13</td>
<td>Original lipase clone obtained by insertion of partial S. exfoliatus M11 DNA in the BamHI site of pJ1486</td>
<td>34</td>
</tr>
<tr>
<td>pB28</td>
<td>Insert from pB13 cloned as a BglII fragment in the single BamHI site of pJ1903</td>
<td>This work</td>
</tr>
<tr>
<td>pB36</td>
<td>Entire insert from pB13 taken out as an XbaI-SsrI fragment, blunt-ended with Klenow, and cloned into SmaI-cut pIJ2925</td>
<td>This work</td>
</tr>
<tr>
<td>pB47</td>
<td>Obtained by religating BlsEII-XhoI-cut pB36 after blunt-ending with Klenow. Carries only lipA with associated promoter region</td>
<td>This work</td>
</tr>
<tr>
<td>pB48</td>
<td>Mel' Hyg' vector derived from the pJV1 minimal replicon</td>
<td>39</td>
</tr>
<tr>
<td>pB49</td>
<td>2.1-kb ApaI fragment carrying a promoterless lipA gene cloned into the SmaI site of the pJ2925 polylinker; transferred as an BglII fragment into pIJ702 following insertion of a SsrI fragment carrying dogA, lipR, and dogA promoters in front of lipA</td>
<td>This work</td>
</tr>
<tr>
<td>pB51</td>
<td>Insert from pB47 cloned as an EcoR1-HindIII fragment in pJ486. Carries only lipA with its associated promoter region</td>
<td>This work</td>
</tr>
<tr>
<td>pB72</td>
<td>Derivative of pIJ2925 with modified polylinker from pUCBM21 (Boehringer Mannheim)</td>
<td>This work</td>
</tr>
<tr>
<td>pB94</td>
<td>Derivative of plJ486 with lipA and lipR genes but not orf481. The TTA codon inside lipR has been replaced by a CTC codon. Same orientation as in pB13</td>
<td>This work</td>
</tr>
<tr>
<td>pB96</td>
<td>Same as pB94 but retains the TTA codon inside lipR</td>
<td>This work</td>
</tr>
<tr>
<td>pB98</td>
<td>Derivative of pB72 carrying the 364-bp HindIII-SsrI fragment of pB13, containing part of the plJ486 polylinker, the lipA promoter region, and the N-terminal part of lipA up to the first SsrI site</td>
<td>This work</td>
</tr>
<tr>
<td>pB99</td>
<td>Derivative of pJ6021 with lipR cloned under the control of the tipA promoter</td>
<td>This work</td>
</tr>
<tr>
<td>pB100</td>
<td>Insert from pB47 cloned as a BglII-PstI fragment into pB48</td>
<td>This work</td>
</tr>
<tr>
<td>pB104</td>
<td>Derivative of pJ6021 with lipA cloned under the control of the tipA promoter</td>
<td>This work</td>
</tr>
<tr>
<td>pJ1486</td>
<td>High-copy-number Streptomyces cloning vector, derived from the pIIH1 replicon</td>
<td>46</td>
</tr>
<tr>
<td>pJ702</td>
<td>High-copy-number Streptomyces cloning vector, derived from the pIIH1 replicon</td>
<td>20</td>
</tr>
<tr>
<td>pJ903</td>
<td>E. coli-Streptomyces shuttle vector derived from SCP6 <em>E. coli</em> origin of replication, pB98 [Table 1] was cut with <em>Bgl II</em> and <em>Sst I</em> and ligated with its associated polylinker, the <em>Bgl II</em> site of the pIJ2925 polylinker; the <em>Sst I</em> site of the pIJ2925 polylinker; the <em>Sst I</em> site of the pIJ2925 polylinker</td>
<td>This work</td>
</tr>
<tr>
<td>pJ925</td>
<td>Derivative of pUC18 with modified polylinker flanked by BglII sites</td>
<td>27</td>
</tr>
<tr>
<td>pJ6021</td>
<td>Kan′ high-copy-number inducible expression vector for Streptomyces, derived from the pIIH1 replicon and carrying the strong and inducible tipA promoter</td>
<td>44</td>
</tr>
</tbody>
</table>

RESULTS

The cloned S. exfoliatus M111 lipA gene directs growth phase-dependent lipase synthesis in S. lividans. We have previously reported that when _S. lividans_ 1326 carrying the cloned extra-cellular lipase gene (lipA) from _S. exfoliatus_ M11 in a high-copy-number plasmid (pB13 [Table 1]) is grown in rich Luria-Bertani medium, lipase accumulation is observed and large amounts of lipase protein can be recovered from the culture supernatant (34). When the timing of lipase synthesis by these cultures was analyzed more carefully, we found that most of the lipase activity appeared in the medium once the culture had entered stationary phase. As can be seen in Fig. 1, very little lipase activity was found in the medium during exponential growth but lipase activity increased constantly once the cultures entered stationary phase. Gel electrophoresis of total protein from 1326(pB13) culture supernatants (Fig. 2A) re-

**TABLE 1. Plasmids used in this study**

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revealed no significant amounts of extracellular proteins by 24 h, which usually corresponded to early exponential phase. By 48 h, which corresponded to early stationary phase, most proteins in the supernatant had been secreted, including a polypeptide with an apparent molecular mass of 30 kDa. No significant increase in the amounts of most proteins, and in fact a decrease in some, could be observed at 72 and 96 h, with the exception of the 30-kDa polypeptide, which continued to be secreted during the stationary phase; this protein showed a mobility identical to that of the purified lipase. A Western blotting experiment with anti-lipase polyclonal antibodies showed that the 30-kDa polypeptide, which accumulated during the stationary phase in 1326(pB13) supernatants, indeed corresponded to the lipA-encoded lipase (Fig. 2B); cultures of S. lividans 1326 carrying the vector pIJ486 did not show the presence of this polypeptide at any point of the growth curve (34 and data not shown). Therefore, lipase activity in the culture supernatants provided a reliable indication of the amount of lipase synthesized and secreted into the medium, and it was subsequently used to assay the amount of lipase produced by different cultures. Reliable determination of lipase activity was greatly facilitated by the stability of the lipA-encoded lipase: less than a 10% loss in activity was observed after incubation of crude culture supernatants at 30°C for up to 1 week, and no significant loss was seen at 4°C after several weeks.

Since the observed increase in lipase secretion after cessation of growth could merely reflect an increase in plasmid copy numbers after the cultures entered stationary phase, the insert from pB13 was transferred as a BglII fragment into the BamHI site of pIJ903, an SCP2*-derived vector whose copy number is tightly controlled and maintained at one to two copies per chromosome (3a, 27). When lipase secretion into the medium by S. lividans 1326 carrying this plasmid (pB28) was measured, a pattern of lipase accumulation similar to that of 1326(pB13) was observed: while maximal lipase activity in the medium was about 20 to 30 times lower than that obtained with 1326(pB13) cultures (reflecting the reduced presence of lipA), most of the lipase activity appeared after the cultures entered stationary phase. This result suggested that lipase secretion by cultures of S. lividans carrying the cloned lipA gene was growth phase-dependent and mostly occurred after exponential growth had ceased.

Lipase gene transcription requires the product of an additional gene. Only very small amounts of lipase activity were found in the medium when 1326(pB51) cultures were analyzed (Fig. 3); pB51 carries the lipA gene and promoter but lacks any sequences downstream of lipA that are present in the original pB13 insert. In addition, gel electrophoresis and Western blot analysis of total protein in the supernatant of 1326(pB51) cultures failed to reveal a significant amount of lipase polypeptide (Fig. 2). This confirmed our previous observation that lipA expression requires additional DNA sequences present in pB13 (34). This requirement could be at the transcriptional level, if lipA could only be transcribed from its own promoter in the presence of some additional factor encoded in pB13, or at the posttranscriptional level if additional factors were re-
required for translation of the lipA message or for lipase processing and/or secretion. In order to determine which was the case, the lipA gene was placed under the control of the regulatory regions of other streptomycete genes. In particular, lipA was placed under the transcriptional control of the dagAp3 and dagAp4 promoters (4) in pB49 and under the transcriptional and translational control of the tipA regulatory region (15, 30) in pB104. These different plasmids were introduced into S. lividans 1326, and the temporal pattern of lipase secretion into the medium was analyzed. As can be seen in Fig. 3, lipase secretion into the medium occurred in all cases. In addition, it is possible to see that these constructs differ both in the amount of lipase secreted into the medium and in the temporal pattern of expression. In 1326(pB49) cultures lipase secretion into the medium coincided with the exponential growth phase; the amount of lipase secreted, however, was lower than that observed when its own promoter in pB13. Higher lipase levels, similar to those found in 1326(pB13) cultures, were obtained in 1326(pB104). These different plasmids were introduced into S. lividans 1326 cultures carrying different plasmids (Fig. 5). Analysis of the DNA sequence revealed the presence of two downstream open reading frames (ORFs) in addition to lipA (Fig. 6); both of these ORFs would be transcribed in the same orientation as lipA. The start of the first ORF (orf934, encoding a protein of 934 amino acids) starts just downstream of the same sequence was found in 1326(pB13) cultures, whereas no transcripts could be detected in 1326(pB51) RNA preparations. Interestingly, lipA transcripts could only be detected in RNA preparations of 1326(pB13) cultures obtained during the stationary phase. Therefore, lipA transcription requires some factor encoded in pB13 and appears to be growth phase dependent.

Sequence of the pB13 insert reveals a gene encoding a potential transcriptional activator. To identify any factor necessary for efficient lipA transcription, the sequence of the entire pB13 insert was obtained (Fig. 5). Analysis of the DNA sequence revealed the presence of two downstream open reading frames (ORFs) in addition to lipA (Fig. 6); both of these ORFs would be transcribed in the same orientation as lipA. The start of the first ORF (orf934, encoding a protein of 934 amino acids) had already been observed when the lipA sequence was reported (34); it starts 399 bp downstream of lipA and ends just before the rightmost KpnI site of the insert (at nt 4450 [Fig. 6]). The second ORF (orf481, encoding a protein of 481 amino acids) starts just downstream of the same KpnI site and ends a few nucleotides upstream of the right-hand end of the pB13 insert (Fig. 6). In order to find whether the product of either
one or both of these ORFs was required for lipA transcription, a series of deletions were obtained with the help of exonuclease III, starting at the right-hand end of the pB13 insert; plasmids carrying these deletions were introduced by transformation into S. lividans 1326, and lipase production by these cultures was quantified and compared to that of 1326(pB13) and 1326(pB51) cultures. S. lividans 1326 carrying plasmids with deletion endpoints located to the right of the KpnI site at nt 4450 (which removed most of orf481) secreted lipase at about the same levels as cultures carrying pB13, whereas cultures carrying plasmids with deletion endpoints to the left of the same KpnI site (which removed the C-terminal part of orf934) showed a dramatic decrease in lipase synthesis, comparable to that observed for 1326(pB51) cultures (Fig. 6). Therefore, only the product of orf934 appeared to be required for lipA transcription, and it was accordingly renamed lipR.

Analysis of the lipR sequence revealed an extremely high guanine-plus-cytosine (G + C) content: an average of 80.2 mol% throughout the length of the gene, which is significantly higher than the average 74 mol% of most Streptomyces genes.
and higher than that of any of the sequences analyzed by Wright and Bibb (48). This is due to an unusually high G+C content in the first and second positions of codons (84.5 and 63.6 mol%, respectively), whereas the third position of codons has the high G+C content expected for a Streptomyces gene (92.4 mol%). This very high G+C content is due to the high proportion of amino acids whose codons have either G or C in the first and second positions: alanine (23.3 mol%), arginine (10.4 mol%, of which only 2 of 98 codons are AGG), and glycine (10.3 mol%). The LipR sequence also contains a large number of leucine residues (13%), of which one is encoded by the rare TTA codon. Analysis of the LipR sequence with the REGULAT program (PC/Gene release 6.85) revealed the presence of a helix-turn-helix motif close to the C terminus of the protein; this program compares sequences with separate weight matrices obtained from helix-turn-helix motifs of positive and negative regulatory proteins, and LipR scored as a likely positive regulator. In addition, when the weight matrix proposed by Dodd and Egan (7) was used to analyze the LipR sequence it gave a greater than 90% probability of LipR being a DNA-binding protein (corresponding to a score of 4.2 standard deviations). A BLAST search (1) was conducted on the

FIG. 5—Continued.
LipR sequence; the only high-scoring sequence obtained in this search was the product of a gene that has been only partially sequenced and that is located just downstream of the *S. albus* *GlipA* homolog (GenBank accession no. U03114). In addition, the BLAST search revealed a large number of protein sequences which gave a lower, but significant, local alignment score; in all cases it was the same region of LipR which was responsible for the local alignment, and this was precisely...
the C-terminal region which includes the helix-turn-helix motif. Most of these proteins are known response regulators, whereas others are transcriptional activators which possess evolutionarily related C-terminal DNA-binding domains and lack the receiver module of response regulators (33a). These proteins have been grouped together, based on the similarity of their DNA-binding domains, in the UhpA/LuxR superfamily of regulators as defined in the PROSITE database (2, 11a). The GenBank or Swiss-Prot accession numbers for the different sequences are U85412 (Rhodococcus sp. BpdT), U10553 (K. pneumoniae AcoK), AE000418 (E. coli MalT), D49928 (Klebsiella aerogenes MoaR), Y00859 (B. subtilis LuxR), P10958 (Rhodococcus sp. BpdT), P11470 (B. subtilis GerE). The helix-turn-helix motif identified in LipR by the REGULAT program is boxed.

**FIG. 7.** Alignment of the C-terminal putative DNA-binding domain of LipR with the homologous domains of other known transcriptional activators. Positions showing strong conservation are shaded. The consensus shown below the aligned sequences is that of the class 3 family of response regulators (33a) and associated proteins, which is equivalent to the UhpA/LuxR superfamily of regulators as defined in the PROSITE database (2, 11a). The GenBank or Swiss-Prot accession numbers for the different sequences are U85412 (Rhodococcus sp. BpdT), U10553 (K. pneumoniae AcoK), AE000418 (E. coli MalT), D49928 (Klebsiella aerogenes MoaR), Y00859 (B. subtilis LuxR), P10958 (Rhodococcus sp. BpdT), P11470 (B. subtilis GerE). The helix-turn-helix motif identified in LipR by the REGULAT program is boxed.

**FIG. 8.** Transcription of lipA in *S. coelicolor* J1501 (lipA*) and J1700 (lipA*) carrying different plasmids. Plasmids pB94 and pB96 are identical constructs which carry only the lipA and lipR genes; in pB94 the single TTA codon inside lipR had been replaced by another leucine codon (CTC) by means of site-directed mutagenesis (plasmid pB94 [Table 1]); an identical plasmid that carried the wild-type (TTA-containing) lipR sequence was constructed as a control (pB96 [Table 1]). Both plasmids were introduced into *S. coelicolor* J1501 and J1700, and lipase production was assayed qualitatively on olive oil-rhodamine B plates; as expected, J1501 carrying either plasmid showed halos of lipolysis, whereas only J1700 (pB94) was lytic. The temporal pattern of lipA transcription by these four strains was analyzed; as seen in Fig. 8, abundant lipA transcripts were found in RNA preparations from stationary-phase cultures of both J1501 (pB94) and J1501 (pB96), and this correlated with lipase activity appearing in the medium only during stationary phase (data not shown). lipA transcription by J1700 (pB94) cultures and lipase activity in the supernatant were also mainly restricted to the stationary phase (Fig. 8); on
plored exposure of the autoradiographs a small number of lipA transcripts could be detected in exponential-phase RNA preparations from J1501 cultures carrying either plasmid and from J1700(pB94) cultures (data not shown). On the other hand, J1700(pB96) cultures were unable to secrete lipase and no lipA transcripts could be detected in either exponential- or stationary-phase RNA preparations from these cultures, even after prolonged exposure of the autoradiographs. This experiment confirmed that lipA transcription is dependent on the lipR product.

Replacement of the TTA codon by the alternate CTC codon in lipR alleviated bldA dependence but not the apparent growth phase dependence of lipA transcription (Fig. 8); this result could be explained if transcription of lipR were itself growth phase dependent and mostly restricted to the stationary phase. In order to find out whether this was the reason for delayed expression of lipA in cultures carrying pB94, the version of lipR lacking the TTA codon was placed under the control of the tipA promoter region, after the introduction of a suitable NdeI site at the start codon in order to allow cloning into the expression vector pIJ6021, which resulted in plasmid pB99. This plasmid was introduced into S. lividans 1326 carrying pB100, which consists of the pJ1V1-derived vector pB48 (conferring hygromycin resistance [39]) into which the lipA gene and promoter had been cloned in the absence of any lipR sequences (Table 1). As a control, pIJ6021 was also introduced into S. lividans 1326(pB100). S. lividans 1326 carrying both pairs of compatible plasmids was assayed for lipase production on olive oil-rhodamine B plates; colonies of S. lividans carrying pIJ6021 plus pB100 were unable to form halos of lipolysis, whereas those carrying pB99 plus pB100 showed large fluorescent halos, indicating that the lipR product was capable of activating lipA transcription in trans. Liquid cultures of S. lividans 1326(pB99/pB100) revealed that lipase was secreted from the start of the exponential growth phase and that addition of thiostrepton significantly increased the amount of lipase in the medium, most of which accumulated during the exponential growth phase (Fig. 9); as expected, control cultures of 1326(pIJ6021/pB100) were unable to secrete lipase, irrespective of lipA induction.

DISCUSSION

Even though a significant number of prokaryotic lipase genes have been cloned (18), there have been few studies aimed at elucidating the molecular mechanisms which regulate their expression; there is, however, evidence that some lipase genes are subject to transcriptional regulation. Thus, in Staphylococcus aureus S6C a chromosomal mutation in the xpr locus, unlinked to the lipase structural gene, eliminated extracellular lipase production (40); the effect of this mutation was shown to be at the transcriptional level, although it was not specific, since transcription of several genes encoding exoproteins was affected (13). In addition, gene fusion experiments have shown that lipase synthesis in Acinetobacter calcoaceticus BD413 is regulated at the transcriptional level, and the existence of a regulator for lipase gene transcription has been inferred, although it has not yet been identified (21). The results presented in this paper show that transcription from the S. exfoliatus M13 lipA promoter requires the presence of a contiguous downstream gene, lipR, since lipA transcripts could be detected only in its presence; this requirement appeared to be specific for transcription from the lipA promoter, since it was overcome when lipA was fused to the promoter regions of other streptomycete genes. The presence of a TTA codon in lipR allowed us to show that lipA transcription was dependent on the LipR protein, since no transcripts could be observed in a S. coelicolor BldA^- mutant when the wild-type bldA-dependent lipR gene was present, whereas normal transcription was observed in the presence of a bldA-independent version of lipR; in addition, lipR provided in trans directed high levels of lipase synthesis which responded to the timing of controlled lipR induction (Fig. 9). The most relevant characteristic of the LipR sequence is the presence of a putative DNA-binding domain at its C terminus; sequence comparison reveals that this domain shows significant similarity to the highly conserved C-terminal DNA-binding domains of a large family of proteins which comprises mostly, but not exclusively, response regulators (2, 33a). Other members of this family include transcriptional activators with a homologous DNA-binding domain that lack the receiver module of response regulators, such as members of the LuxR subfamily of cell density-responsive regulators (11a) as well as other transcriptional activators, such as MalT; in the case of MalT it has been shown that this C-terminal domain is sufficient for DNA binding (44a). MalT was the only activator belonging to this family with a disparate, larger size (33a) until the recent description of AcoK, a MalT homolog of similar size (33b) which also functions as a transcriptional activator. LipR shows significant similarity to these two activators which is not restricted to the DNA-binding domain. Therefore, LipR appears to be the third member of a subfamily of proteins of similar size which belong, based on their DNA-binding domains, to the UhpA/LuxR superfAMILY of regulators (2, 33a). All these lines of evidence support the notion that lipR encodes a transcriptional activator of the lipA promoter. The presence of a gene encoding a LipR homolog downstream of the lipA gene of S. albus G suggests that the close physical linkage of lipase genes and their cognate regulatory genes in Streptomyces might be common; in fact, Southern hybridization experiments

![Figure 9](https://example.com/figure9.png)
have revealed sequences homologous to lipR in the same Streptomyces strains previously shown to hybridize to a lipA-specific probe (6) but not in strains lacking lipA-homologous sequences (unpublished results). Given the close physical association of the lipA and lipR genes, LipR is likely to be a specific regulator of lipase synthesis rather than a general one, as in the case of the S. aureus regulator encoded in the unlinked xpr locus (13). Therefore, the identification and sequencing of LipR appears to be the first report of a specific transcriptional regulator of lipase gene expression in prokaryotes.

The results presented here show that transcription of lipA is growth phase dependent under the conditions used and that this growth phase dependence is mediated by lipR. The apparent growth phase dependence was evidenced by the increase in lipase activity and protein during stationary phase, which correlated with the observation that lipA transcripts were abundant only in RNA preparations obtained during the stationary phase; it should be noted, however, that there are other possibilities which could account for these observations. An increase in the plasmid copy number upon entry into stationary phase, for example, could lead to similar results. This possibility is unlikely, however, since it has been shown that the copy number of pIJ101-based vectors in batch culture conditions is not significantly altered after entry into the stationary phase (in fact copy number is higher during the exponential growth phase [48a]); these changes could hardly account for the difference in the number of lipA transcripts between exponential- and stationary-phase cultures; in addition, growth phase-dependent lipase synthesis was also observed with an SCP2*-derived vector, whose copy number is tightly controlled and maintained at one to two copies per chromosome, even in stationary-phase cultures (3a). Another possible explanation for the results obtained in this work could be that a change in the stability of lipA transcripts, rather than growth phase-dependent initiation of transcription at the lipA promoter, is responsible for their increased abundance during stationary phase; if this were so, then the stabilization of lipA transcripts should be mediated by LipR. This is an unlikely possibility, however, since LipR is a homolog of known transcriptional activators with a well-conserved DNA-binding domain.

Since expression of the S. exfoliatus M11 lipase is bldA dependent, a parallel can be drawn to the expression of genes encoding biosynthetic enzymes of the actinorhodine and undecylprodigiosin pathways of S. coelicolor A3(2) (3, 12, 43); in these two well-studied cases, specific transcriptional activators (the products of the actII-ORF4 and redD genes) turn on their respective pathway-specific genes during the transition into stationary phase, since their genes are in turn transcribed in a growth phase-dependent fashion (12, 43) in response to an as yet unidentified signal (3). In the case of actinorhodine, bldA dependence is caused by the presence of a TTA codon in actII-ORF4 (9), whereas in the case of undecylprodigiosin, bldA dependence is mediated by RedZ, an additional activator of redD transcription. The redZ gene has a TTA codon (47). Interestingly, the RedZ sequence (GenBank accession no. Y07902) reveals a putative DNA-binding domain similar to that of LipR (Fig. 7), and the position of the TTA-encoded leucine in both sequences is close to the C terminus (in the case of RedZ it is inside the DNA-binding domain, and in the case of LipR it is a few amino acids before it). The bldA dependence of LipR does not appear to be the main cause for growth phase-dependent lipase expression, since replacement of the single TTA codon in lipR by an alternate leucine codon resulted in the same temporal pattern of lipA transcription; this is in agreement with the observation that the timing of actII-ORF4 and redZ transcription, rather than their bldA dependence, is the main cause for growth phase-dependent actinorhodine and undecylprodigiosin synthesis (12, 47). Clearly, it will be necessary to study lipR transcription in more detail in order to confirm whether its transcription is growth phase dependent and to explore the possibility that it shares other regulatory aspects with actII-ORF4 and redD, such as in vitro transcription by RNA polymerase holoenzyme carrying αredD (11) or conditional dependence on the product of the pleiotropic gene afsR (10).

Why should lipase expression be limited to the stationary phase? One possible explanation might be related to the recent finding that Streptomyces spp. are perhaps unique among prokaryotes in using triacylglycerols as storage compounds; it has been speculated that these serve as possible carbon sources for antibiotic synthesis (32). These triacylglycerols are accumulated intracellularly in membrane-bound structures during the exponential growth phase (33), and it is clear that the first step in their utilization should involve lipolytic enzymes. The fact that the Streptomyces lipases are extracellular does not necessarily mean that triacylglycerols accumulated intracellularly might not be available to them, since under normal conditions for Streptomyces growth (surface-grown cultures) lysis and cannibalism of the substrate mycelium play roles in providing the differentiating and antibiotic-producing parts of the colonies with nutrients (5).

In conclusion, we have shown that transcription of the cloned S. exfoliatus M11 lipA gene is bldA dependent and that this dependence is mediated by a putative transcriptional activator of the lipA promoter, encoded by the lipR gene downstream of lipA, which has a single TTA codon. The apparent growth phase-dependent expression of lipA is not a consequence of bldA dependence and is also mediated by LipR.

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