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# Identification of a Protein-Binding Sequence Involved in Expression of an Esterase Gene from *Streptomyces scabies*

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**Expression of an esterase gene from *Streptomyces scabies* is regulated by zinc in both *Streptomyces scabies* and *Streptomyces lividans*. A specific protein-binding site was identified on an esterase promoter fragment by using an S-30 extract from *S. scabies*. The location of the protein-binding site was determined by gel shift assays of promoter deletion fragments and by DNase I footprinting analysis. The protein-binding site maps from nucleotides −59 to −81 relative to the start of transcription. An esterase gene construct cloned and expressed in *S. lividans* was used to assess the importance of the protein-binding site. Deletion of the 23-bp protein-binding site resulted in a 10-fold decrease in esterase production when cells were grown in zinc-inducing conditions. The protein-binding site may represent a region involved in positive regulation of the *S. scabies* esterase gene.**

The gram-positive soil bacterium *Streptomyces scabies* is the causative agent of scab disease which affects a variety of underground vegetables including the potato (5). The underground portions of these plants are coated with a waxy polyester, suberin, which acts to control moisture loss and perhaps acts as a barrier to invasion by plant pathogens (15, 16). Invasion by the pathogen induces the proliferation of suberin by the host at the site of the infection. Enzymes involved in the breakdown of suberin or other components on the surface of the potato tuber may play an important role in invasion by *S. scabies*.

In previous studies, it was demonstrated that pathogenic isolates of *S. scabies* produce heat-stable extracellular esterases when grown in minimal medium supplemented with suberin extracted from potato peel (21). Zinc associated with the suberin was identified as the critical component involved in the induction of esterase activity. When *S. scabies* was grown in minimal medium with the addition of suberin or 2.0 μM $Zn(SO_4)_2$, esterase activity was induced 20- to 50-fold (21). The esterase gene from the pathogenic *S. scabies* isolate FL1 has been cloned and sequenced, and the transcriptional start site has been determined by nuclease S1 mapping (24). The amount of esterase transcript detected from *S. scabies* FL1 cultures grown in the presence of zinc was greatly enhanced compared with that from cells grown without zinc. The results have suggested that zinc regulation of the esterase gene may be at the level of transcription. One model consistent with the data involves positive regulation of esterase gene transcription mediated by the binding of a regulatory protein. Zinc may be involved in the binding and/or activation of this regulatory protein.

In this article we report the identification of a specific protein-binding site which is 5′ proximal to the esterase promoter sequence. Site-directed deletion analysis has been used to demonstrate the importance of this protein-binding sequence to the regulation of esterase gene expression.

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## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *S. scabies* FL1, a pathogen of the potato, was isolated from a scab lesion as described previously (20). The esterase gene was subcloned as a 2.5-kb *Sma*I fragment into the *Sma*I site of pUC119 (33). The clone containing the esterase gene in the same orientation as *lacα* is pJW27; the construct with the esterase gene in the opposite orientation is pJW9 (24). Deletion subclones of the esterase gene were made in pJW9 or pJW27 (24). *Escherichia coli* JM109 (35) was used to maintain *E. coli* plasmids. The esterase gene was transformed into *Streptomyces lividans* TK24 (13) with streptomycete vector pIJ486 (34).

**Media and growth conditions.** *S. scabies* FL1 was grown on oatmeal agar (28) at 30°C and maintained as spore suspensions in 20% glycerol at −20°C. *S. scabies* cultures used in an S-30 cell lysate preparation were grown in NMM minimal medium, a modification of NMMP medium which does not contain polyethylene glycol (12). Esterase induction requires the addition of 2.0 μM $Zn(SO_4)_2$. All liquid cultures were grown at 30°C with shaking in flasks containing springs.

*S. lividans* TK24 was grown on R2YE agar medium and maintained as spore suspensions in 20% glycerol at −20°C (12). For selection of resistant *S. lividans* transformants containing pIJ486 derivatives, thiostrepton was incorporated into solid medium at 40 μg/ml and liquid medium at 5 μg/ml (12).

*E. coli* JM109 was grown on antibiotic medium 2 agar plates (Difco Laboratories, Detroit, Mich.) and in LB (26) liquid cultures at 37°C. For selection of JM109 transformants containing pUC119 and derivatives, ampicillin was incorporated into the medium at 50 μg/ml (26).

**DNA manipulations and bacterial transformations.** Plasmid DNA was isolated as previously described (1). Restriction enzymes were obtained and reactions were carried out according to the manufacturer's instructions (Gibco Bethesda Research Labs, Gaithersburg, Md., and New England Biolabs, Beverly, Mass.). DNA restriction fragments were electrophoresed on a 1.0% agarose gel with Tris-borate-EDTA buffer (pH 8.0; TBE buffer) (26) and purified by electroelution with an electroelution apparatus (Interna-

tional Biotechnologies Inc., model UEA) according to the manufacturer's specifications.

Exonuclease III deletions from the 5′ side of the esterase gene into the promoter region were made by the method of Henikoff (11) by using a *Kpn*I-*Nru*I digest of pJW9 (24). The subclones were named U1, U2, U3, U4, and U5, and the endpoints of the deletions were mapped by dideoxy sequencing (17, 27) (see Fig. 1).

Protoplast preparations and transformations of *S. lividans* TK24 were performed as described by Hopwood et al. (12). Competent cells prepared with $CaCl_2$ were used for all *E. coli* JM109 transformations (8).

**S-30 extract preparation.** S-30 cell extracts of *S. scabies* FL1 were prepared by the in vitro transcription-translation system of Calcutt and Cundliffe (3). *S. scabies* was grown in 100 ml of NMM medium with 2.0 μM $Zn(SO_4)_2$ to the early stationary phase and harvested by centrifugation at 12,000 × *g* for 10 min. All succeeding steps were carried out at 4°C. The cells were washed twice in suspension buffer (10% [vol/vol] glycerol, 10 mM magnesium acetate, 1.0 M KCl, 10 mM β-mercaptoethanol, 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid]-KOH [pH 7.6]) and then once in cracking buffer (10% glycerol, 10 mM magnesium acetate, 60 mM $NH_4Cl$, 5.0 mM β-mercaptoethanol, 50 mM HEPES-KOH [pH 7.6]). The cells were resuspended in cracking buffer and lysed by one pass through a French pressure cell at 12,000 lb/in$^2$. Cell debris was removed by centrifugation twice for 30 min at 30,000 × *g*. The S-30 supernatant was stored in aliquots at −80°C. The S-30 extract was used for gel retardation assays and DNase I footprinting experiments.

**Gel retardation assays.** Protein-DNA binding was measured by a gel retardation assay (25). The reaction mixture contained 750 ng of dIdC DNA (Pharmacia LKB Biotechnology, Piscataway, N.J.), 0.75 μg of bovine serum albumin, 50 mM KCl, 1.0 mM $MgCl_2$, 12 mM HEPES-KOH (pH 7.4), 5.0 mM dithiothreitol, 0.5 ng of radiolabelled fragment, 10% (vol/vol) glycerol, and 5.0 μg of S-30 extract in a total volume of 15 μl. The reaction mixture was incubated for 30 min at 30°C and loaded directly on a 4.0% polyacrylamide gel with 0.5× TBE buffer. The gel was run at 300 V, dried, and subjected to autoradiography.

The *Eco*RI DNA fragments used for these assays were electroeluted from 1.0% agarose or 2.0% Nusieve GTG agarose (FMC, Rockland, Maine) gels. The *Eco*RI ends of the purified fragments were filled in with Klenow enzyme and [α-$^{32}$P]dATP (800 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) (26).

**DNase I footprinting.** DNase I footprinting was carried out with an esterase gene fragment from pJW9. The plasmid was digested with *Eco*RI, the noncoding strand was end labelled in a reaction mixture containing T4 polynucleotide kinase (Gibco Bethesda Research Laboratories) and [γ-$^{32}$P]ATP (6,000 Ci/mmol; Amersham), or the 5′ overhang was filled in with Klenow enzyme and [α-$^{32}$P]dATP (800 Ci/mmol; Amersham) according to Sambrook et al. (26) to label the coding strand. After a second digestion with *Nru*I, the *Eco*RI-*Nru*I fragment was gel purified. The protein-DNA binding reaction was carried out as for the gel retardation assays with various amounts of S-30 extract. After the 30-min binding reaction, 0.05 U of RQ1 DNase I (Promega Corp., Madison, Wis.) was added. The reaction mixture was incubated for 2 min at 30°C, and the reaction was stopped by the addition of phenol-chloroform and vortexing. The DNA was ethanol precipitated, denatured in formamide loading buffer (26), and run on a 6% polyacrylamide-urea denaturing gel (26). Maxam-Gilbert sequencing reactions of the end-labelled fragment (19) or dideoxy-sequencing reactions of pUC119 with universal primer (17, 27) were used as DNA size standards. After electrophoresis, the gels were dried and subjected to autoradiography.

**Site-directed mutagenesis.** The pJW27 plasmid was used to construct a deletion of the protein-binding sequence from nucleotide (nt) −58 to nt −81 relative to the transcriptional start site in the esterase gene by using the oligonucleotide 5′GGTGATCAAACCGGGGTGTCCATCTCACC3′. Site-directed mutagenesis was performed by the methods of Kunkel et al. (18) by using the Muta-Gene in vitro mutagenesis kit (Bio-Rad Laboratories, Richmond, Calif.). The deletion of the protein-binding region was confirmed by sequence analysis of the resulting construct, pJLS129.

**Esterase assay and protein determination.** Esterase activity was measured in culture filtrates by a spectrophotometric assay with *p*-nitrophenyl-butyrate as the substrate (21). All cell filtrates were heat treated at 65°C for 30 min to inactivate heat-labile esterases (21). The protein concentration of the S-30 extracts was determined by the method of Bradford (2).

## RESULTS

**Evidence for a specific protein-binding site.** The first approach to identify regions of the esterase gene important in the regulation of expression was to generate deletions in the promoter-proximal sequence. Deletions were made in the esterase gene with exonuclease III after digestion of pJW9 with *Kpn*I and *Nru*I (24). The resulting deletion subclones are shown in Fig. 1. The precise endpoints of these deletions were determined by DNA sequencing. The *Eco*RI site within the polycloning region and the *Eco*RI site in the esterase coding sequence were used to isolate purified DNA fragments containing the various-length deletions. *Eco*RI fragments of the deletion constructs U1 to U5 were used in experiments to localize a protein-binding site that was postulated to be necessary for the expression of the *S. scabies* esterase gene.

*S. scabies* was grown in NMM with 2 μM $Zn(SO_4)_2$ to the late exponential phase to prepare S-30 cell extracts for gel retardation experiments. The S-30 extract was used to demonstrate that a protein produced by *S. scabies* binds to a specific region of the esterase sequence. When the $^{32}$P-labelled U1 *Eco*RI fragment (Fig. 1) was incubated with the S-30 extract (Fig. 2A, lane 1), its migration on a polyacrylamide gel was retarded when compared with the migration of the unbound fragment (Fig. 2A, lane 6). The protein-binding activity was detected in cell lysates prepared from *S. scabies* cultures grown with or without 2.0 μM zinc.

To demonstrate that the interaction of the protein with the esterase sequence is specific, increasing amounts of unlabelled U1 *Eco*RI fragment were added to the DNA-protein-binding reaction mixtures (Fig. 2A, lanes 2 to 5). The unlabelled fragment competed with the labelled fragment for binding by the protein. Protein binding to the labelled fragment was not interrupted by the addition of nonspecific dIdC DNA equal to the amount of unlabelled competitor DNA that was added in the experiment whose results are shown in panel A (Fig. 2B).

**Location of the protein-binding site.** To determine the location of the protein-binding site within the sequence 5′ to the esterase gene, $^{32}$P-labelled *Eco*RI fragments of deletion constructs U1, U2, U3, U4, and U5 were used in the DNA-protein binding reactions (Fig. 3). During electrophoresis, the migration of the U1, U2, and U3 fragments was

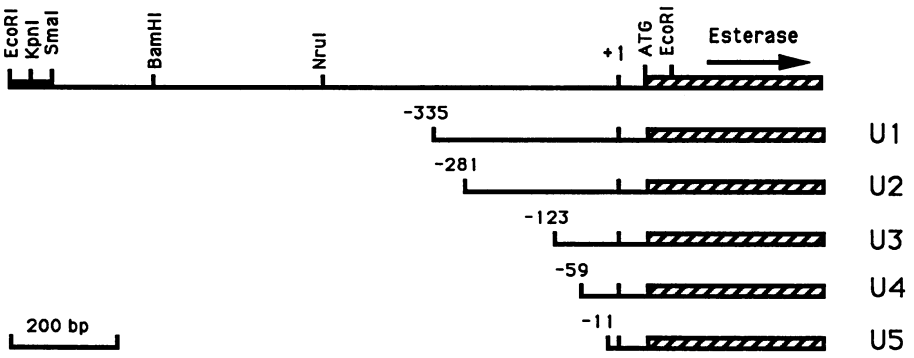


FIG. 1. Restriction map of the *S. scabies* esterase 5′ sequence in pJW9. Exonuclease III-generated deletions were made in the esterase upstream sequence after cleavage with *Nru*I and *Kpn*I. The *Kpn*I site is within the polycloning region in the pJW9 construct. The *Nru*I site is located 556 bp upstream of the esterase gene transcriptional start site. Subclones U1 through U5 contain increasing lengths of deletion. The endpoints of each deletion are numbered relative to the transcriptional start site. The start of transcription is indicated as +1. The hatched boxes represent the beginning of the esterase open reading frame at the ATG codon. The open reading frame extends for another 1,007 bp beyond the *Eco*RI site (24). The closed box represents sites available in the polycloning region of pUC119.

retarded when bound by a protein in the S-30 extract. Migration of the U4 and U5 fragments was not retarded by a protein in the S-30 extract. These results suggest that a specific protein-binding site is located within the U3 fragment but not within the portion of the sequence common to the U4 fragment (Fig. 1).

The specific esterase DNA sequence involved in protein binding was determined by DNase I footprinting experiments (Fig. 4). Either the noncoding or the coding DNA strand was end labelled and incubated with various amounts of the S-30 extract prior to DNase I treatment. The site protected by protein binding was from nt −61 to nt −77 on the noncoding strand (Fig. 4A) and from nt −59 to nt −81 on the coding strand (Fig. 4B) relative to the start of transcription (Fig. 5). A hypersensitive site for DNase I cleavage was observed on the coding strand DNA when bound by S-30 protein. The hypersensitive site was mapped to position −76 (Fig. 4B, lanes 2 to 4). These results are consistent with the gel shift assays in which the *Eco*RI fragments containing the 23-bp protein-binding site (U1 to U3) showed a shift when bound by the protein whereas fragments with the site deleted (U4, U5) did not. The esterase upstream sequence and the location of the DNase I-protected region are shown in Fig. 5.

**Zinc regulation of the *S. scabies* esterase gene in *S. lividans*.** Since an esterase mutant of *S. scabies* was not available, the *S. scabies* esterase gene was cloned and expressed in the nonpathogenic streptomycete *S. lividans* TK24. *S. lividans* TK24 was chosen as the host strain for in vivo studies of esterase gene expression for two reasons. First, *S. lividans* TK24 does not produce a heat-stable esterase when grown under zinc-inducing conditions, thereby facilitating detection of the cloned *S. scabies* esterase (data not shown). Second, gel shift experiments using the U1 and U4 esterase *Eco*RI fragments and S-30 extracts prepared from *S. lividans*

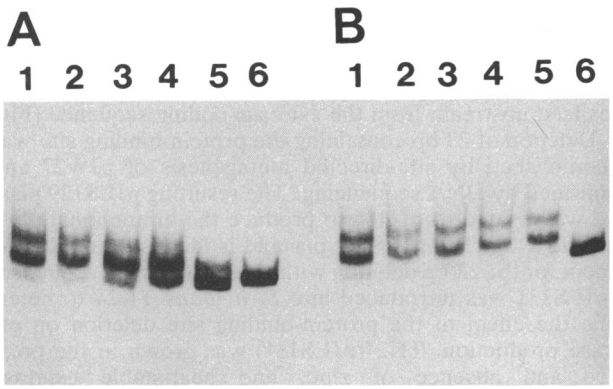


FIG. 2. Gel retardation assay. The DNA-protein-binding reaction mixture contained 0.2 ng of $^{32}$P-labelled U1 *Eco*RI fragment and 5 μg of an S-30 extract prepared from a culture of *S. scabies* grown in NMM containing 2.0 μM $Zn(SO_4)_2$. The reaction mixtures used in panel A contain increasing amounts of competing unlabelled U1 *Eco*RI fragment at 0 (lane 1), 10 (lane 2), 20 (lane 3), 40 (lane 4), and 120 (lane 5) ng. The control reaction mixture used in lane 6 of both panels contained no S-30 extract. The reaction mixtures used in panel B are identical to those used in panel A except that 0 to 120 ng of nonspecific dIdC DNA was added instead of the unlabelled U1 *Eco*RI fragment.

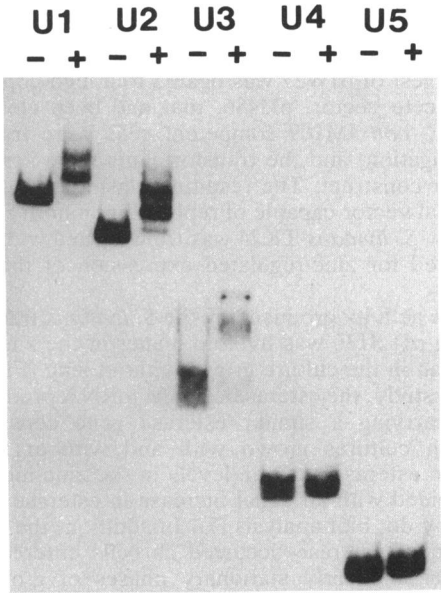


FIG. 3. Localization of the DNA-binding activity. $^{32}$P-labelled *Eco*RI fragments of deletion subclones U1, U2, U3, U4, and U5 were used in gel retardation assays with (+) or without (−) 5 μg of an S-30 extract from *S. scabies*.

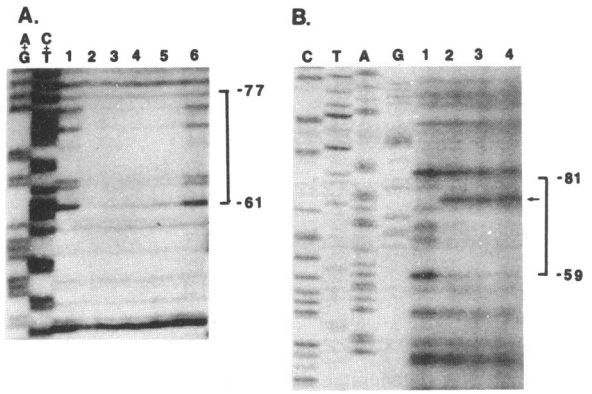


FIG. 4. DNase I footprinting analysis. The DNase I-protected region for both strands of the *S. scabies* esterase regulatory region was determined. The regions bracketed on the gels are portions of the sequence protected from treatment with DNase I. The numbers to the right of the brackets in panels A and B correspond to the nucleotide positions in the sequence relative to the transcriptional start site (see Fig. 5). (A) The *Eco*RI-*Nru*I fragment end labelled at the *Eco*RI site was used to footprint the noncoding strand of the DNA. Protein-DNA binding reactions were carried out with this fragment and 0 (lanes 1 and 6), 50 (lane 2), 25 (lane 3), 10 (lane 4), and 5 (lane 5) μg of S-30 extract. After the binding reaction, the DNA was treated with DNase I and run on a 6.0% polyacrylamide-urea gel. Maxam-Gilbert sequencing reactions (A+G, C+T) were used to identify the DNase I-protected region of the fragment. (B) The *Eco*RI-*Nru*I fragment labelled by filling in the *Eco*RI site with [α-$^{32}$P]dATP was used to footprint the coding strand. Reaction mixtures contained 0 (lane 1), 5 (lane 2), 2.5 (lane 3), and 1 (lane 4) μg of S-30 extract. Sequenase reactions (C, T, A, and G) using universal primer and pUC119 were run to identify the position of the DNase I-protected site (17, 27). The DNase I-hypersensitive site is marked with an arrow.

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                >U1
AGGAGCCTGA CGCGGCCCGA CGCACCGTTG GCAGACTTCC GGCCAAGTAG GCGCAGGCAA

        >U2
CAGCGTCGGC CACGGAACTT GTCTTCGACG GCCGCTGCTC TGTACGGCAG GCCGGCCGCA

TTCCGGACGC CTCCGCCCCG TCGGCACATG CCCACGCCGC CGGGCCCGCG TGGCGGTCGG

                                                    >U3
GCAGGCTCCT CCCCTTTCGG GTCGTGATCA CATCGGTACT CATCGGCCAC ACCTCCCCAG

      -100        -90         -80         -70         -60         -50
                                *                              >U4
CACCCCTGCT GAACAGGTGA GATGGACACA CGTGAGGGGT TGGCCAGGAA CACCCGGTTT
                                 ACTCCCCA ACCGGTCCT

      -40         -30         -20         -10                     10
                                          >U5        +1
GATCACCTCC ATTGGATTAT CACGCTCCGT ATGTGACTGA ATGCAGAGCG TTCCATCTTG

        20          30          40          50          59          68
CTCGGCTCCT CCTCGTGAGC CTCGGTGAAA GGGCACAGCC ATG TCT TCG GCC ATG CGT AAG
                                            MET Ser Ser Ala MET Arg Lys

     77          86          95          104         113         122
ACG ACG AAT TCT CCG GTG GTA CGG CGG CTG ACC GCC GCG GCC GTC GCA CTG GGC
Thr Thr Asn Ser Pro Val Val Arg Arg Leu Thr Ala Ala Ala Val Ala Leu Gly
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FIG. 5. DNA sequence 5′ to the esterase coding region and location of the protein-binding site. The protein-binding sites identified on the coding (nt −59 to nt −81) and noncoding (nt −61 to nt −77) strands are underlined. A DNase I-hypersensitive site is indicated (*) at nt −76. The first nucleotide contained in each of the deletion subclones, U1 to U5, is identified by a caret (>) and the subclone designation. The start of transcription is indicated as +1. The suggested start codon for esterase translation is indicated by the MET at position +51.

TK24 gave results equivalent to those reported for the S-30 extracts of *S. scabies* (Fig. 3). These results indicated that *S. lividans* TK24 produced a similar DNA-binding protein that interacts with the esterase gene.

The esterase gene was originally cloned into pUC119 on a 2.5-kb *Sma*I fragment resulting in plasmid pJW27 (23). A *Hin*dIII digest of pJW27 was ligated to a high-copy-number streptomycete vector, pIJ486, that had been cleaved with *Hin*dIII. *E. coli* JM109 competent cells were transformed with the ligation, and the transformants were screened for the proper construct. The resulting plasmid, pJLS130, is a bifunctional vector capable of replication in both *E. coli* and *S. lividans*. *S. lividans* TK24 was transformed with pJLS130 and assayed for zinc-regulated expression of the esterase gene.

Esterase activity produced by the *S. lividans* transformant containing pJLS130 was fivefold higher in the zinc-induced culture than in the culture grown without zinc (Fig. 6A). In a separate study, the esterase-specific mRNA produced in *S. lividans* carrying a similar esterase gene construct was assayed in cultures grown with and without zinc. The increase in esterase enzyme levels in the zinc-induced culture coincided with an equal increase in esterase transcript detected by dot blot analysis (7). In addition, the maximum production of esterase occurred as cells entered the late exponential and early stationary phases of growth. This timing of esterase synthesis is also similar to that observed in induced *S. scabies* cultures. The fivefold increase in zinc-induced esterase production in the *S. lividans* transformant is not as great as the typical 20-fold induction of esterase by zinc in *S. scabies* cultures (21). However, the evidence that *S. lividans* makes a protein which binds to the esterase DNA fragment together with the evidence for zinc-regulated expression of the esterase gene suggests that *S. lividans* contains the machinery required for zinc regulation of the *S. scabies* esterase gene. Thus, *S. lividans* can be used as an appropriate host strain for studying regulation of the *S. scabies* esterase gene.

**Deletion of the protein-binding site and its effect on esterase gene expression in *S. lividans*.** An esterase gene construct cloned and expressed in *S. lividans* was used to assess the importance of the specific protein-binding site that had been localized upstream from the esterase coding sequence (Fig. 5). Deletion of 23 bp containing the protein-binding site was accomplished by site-directed mutagenesis of pJW27 and confirmed by DNA sequencing. The resulting pJLS129 plasmid was ligated to pIJ486 to produce the bifunctional plasmid pJLS131. The pJLS131 plasmid is identical to pJLS130 except for the 23-bp deletion within the esterase 5′ sequence.

pJLS131 was introduced into *S. lividans* TK24 to determine the effect of the protein-binding site deletion on esterase production. TK24(pJLS131) was grown in the presence and absence of zinc, and heat-stable esterase production was monitored and compared with esterase production by parallel cultures of TK24(pJLS130) (Fig. 6). The pJLS131 transformant produced low levels of esterase when grown without zinc (about 0.2 nmol/min/μl) and showed an approximate twofold increase in activity when the culture was grown in the presence of zinc (Fig. 6B). This twofold increase occurred when the culture reached stationary phase, similar to when normal zinc induction of esterase production is observed (Fig. 6A). However, even the induced esterase levels seen in cells carrying the pJLS131

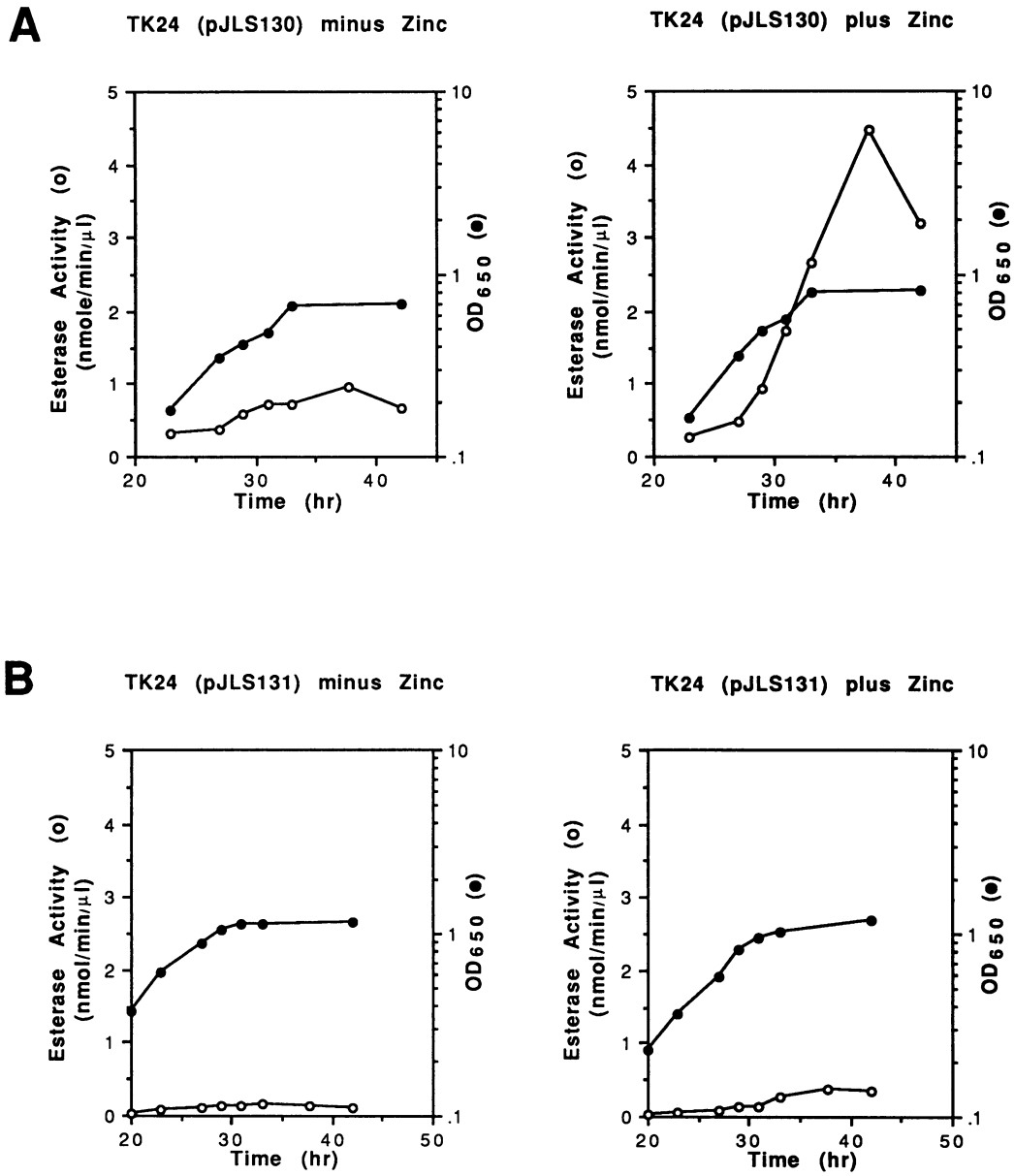


FIG. 6. Zinc-induced heat-stable esterase production in *S. lividans* TK24 containing either pJLS130 or pJLS131. Growth of the culture was monitored by optical density at 650 nm ($OD_{650}$). Heat-stable esterase activity was measured in a spectrophotometric assay and expressed as nanomoles of *p*-nitrophenyl produced per minute per microliter of culture filtrate (21). *S. lividans* strains were grown in NMM minimal medium with or without 2.0 μM $Zn(SO_4)_2$. (A) TK24 containing the entire esterase gene in plasmid pJLS130; (B) TK24 containing the esterase gene with a deletion of the protein-binding region in pJLS131.

construct were at least 10-fold lower than the amount of esterase produced in *S. lividans* containing the intact esterase gene, pJLS130 (Fig. 6A). These results indicate that the 23-bp deletion containing a specific protein-binding site dramatically affects expression of the esterase gene. The protein that binds to this sequence may therefore play an important role in the regulation of esterase gene expression.

## DISCUSSION

Our current model for zinc regulation of esterase gene expression involves the binding of a positive regulatory protein to the promoter region of this gene. In the presence of zinc, this regulatory protein activates transcription of the esterase gene. Gel retardation and DNase I protection experiments have demonstrated that *S. scabies* makes a protein that specifically binds to a nucleotide sequence adjacent to the RNA polymerase-binding site of the esterase gene. The protein-binding activity was detected in extracts prepared from *S. scabies* grown in the presence or absence of zinc (data not shown). Therefore, zinc may not be required for the expression of this binding protein. This protein-binding site plays an important role in esterase gene expression. Deletion of the binding site led to a 10-fold decrease in esterase production.

The addition of zinc to the binding assay was not required for the observed gel shift reaction. It is unclear, however, whether zinc is actually necessary for the protein to bind to the DNA. Either the protein is able to bind to the DNA in the absence of zinc or the buffers used in the S-30 extract contain contaminant levels of zinc sufficient for binding. It is not uncommon for positive regulatory proteins to bind the target gene without the effector present. The regulatory protein of the *mer* operon in *E. coli*, MerR, binds to the *merT* promoter in the absence of mercuric ions (23). The MerR-promoter complex facilitates the binding of RNA polymerase in a closed complex incapable of transcription (6, 10). It is proposed that with the addition of mercuric ions, the MerR protein imposes a conformational change within the promoter sequence resulting in the formation of an open complex with RNA polymerase and the activation of transcription (6, 10). Similarly, the regulatory protein involved in oxidative stress in *Salmonella typhimurium* and *E. coli*, OxyR, binds to the regulatory regions of target genes but is an activator of transcription only when it is in its oxidized form (29).

The protein-binding site identified in the 5′ region of the esterase gene may be involved in general transcriptional activation or in zinc-regulated activation of esterase gene expression. By analogy to MerR (23) and OxyR (29), we propose that the binding of a regulatory protein to this binding site on the esterase DNA is not zinc dependent. The regulatory protein, however, would only act as a positive regulator of transcription in the presence of zinc. The pJLS130 construct contains the normal binding site. When cells containing this construct are grown without zinc, the protein may still bind to the DNA and promote some low-level transcription of the esterase gene. The apparent two-fold increase in esterase activity observed between 25 and 38 h of growth could be accounted for by the approximate twofold increase in cell mass and subsequent accumulation of esterase produced continuously at a low level during this time period. This low-level production from the pJLS130 construct in the absence of zinc is in contrast to the greater-than-fivefold increase in esterase production observed when cells were grown in the presence of zinc.

The pJLS131 construct does not contain the protein-binding site, which would presumably preclude binding of the regulatory protein. When cells with pJLS131 were grown without zinc, esterase synthesis was lower than that observed for cells with pJLS130 grown without zinc. This could be due to the lack of regulatory protein binding to its primary binding sequence. There was an approximate two-fold increase in esterase activity when the deletion construct was grown in the presence of zinc. This suggests that there may be a secondary weak binding site which allows limited protein binding and induction of esterase transcription in the presence of zinc. The protein involved in binding to the esterase gene sequence must be isolated to more fully understand its role in zinc regulation of esterase gene transcription.

The protein-binding site of the esterase gene was localized by gel retardation experiments with nested deletion fragments and by DNase I footprinting of both strands of the esterase DNA. The protein-binding site mapped from nt −61 to nt −77 on the noncoding strand and from nt −59 to nt −81 on the coding strand relative to the start of transcription of the esterase gene. A search of the GenBank data base did not reveal any homology between the esterase protein-binding sequence and other known DNA sequences.

The protein-binding site may represent a positive regulatory region which is 5′ proximal to the esterase RNA polymerase-binding site. The position of the esterase regulatory sequence is similar to the location of the OxyR-binding site relative to the promoter sequence of its target genes (31, 32). The catabolite gene activator protein-binding site within the *lac* operon is also 5′ and adjacent to the RNA polymerase-binding site (30). It has been demonstrated that the stimulation of transcription of the *lac* operon is dependent on protein-protein interaction between the catabolite gene activator protein and the RNA polymerase (30). In *E. coli*, the majority of activator protein-binding sites are proximal to the sigma 70 RNA polymerase-binding site (4). The close proximity of the regulatory protein-binding site and the RNA polymerase-binding site would facilitate interactions between these proteins.

In eucaryotes, zinc is required by several transcription activators which contain zinc finger motifs. The activators do not bind their target DNA and activate transcription in the absence of zinc. Examples of these activators include the *Xenopus* transcription factor IIIA (22), human transcription factor Sp1 (14), and yeast ADR1, which is a regulatory protein required for transcriptional activation of the glucose-repressible alcohol dehydrogenase gene (9). The proposed transcriptional activator involved in the regulation of the esterase gene of *S. scabies* may also be a zinc-binding protein.

Zinc may also be involved in the expression of other genes in *S. scabies*. When *S. scabies* is grown in NMM minimal medium with zinc, a yellow compound is produced in the mycelium (unpublished results). The compound is produced only in the culture grown with zinc, and the appearance of this yellow compound coincides temporally with the production of esterase in *S. scabies*. Even though this yellow compound has not yet been identified, the dependence of its production on zinc suggests that zinc may have a more global effect on gene expression in *S. scabies* and is not limited to regulation of the esterase gene. In addition, expression of the *S. scabies* esterase gene is also regulated by zinc in *S. lividans*, indicating that the mechanism by which zinc regulates gene expression can function in other streptomycetes and is not limited to *S. scabies*. To further characterize regulation of the *S. scabies* esterase gene, regulatory mutants defective in zinc induction will be isolated, and the putative transcriptional activator protein will be purified and further characterized.

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