

Direct repeat sequences in the *Streptomyces* chitinase-63 promoter direct both glucose repression and chitin induction

(transcriptional regulation/catabolite control/carbohydrate utilization)

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ABSTRACT The *chi63* promoter directs glucose-sensitive, chitin-dependent transcription of a gene involved in the utilization of chitin as carbon source. Analysis of 5' and 3' deletions of the promoter region revealed that a 350-bp segment is sufficient for wild-type levels of expression and regulation. The analysis of single base changes throughout the promoter region, introduced by random and site-directed mutagenesis, identified several sequences to be important for activity and regulation. Single base changes at -10, -12, -32, -33, -35, and -37 upstream of the transcription start site resulted in loss of activity from the promoter, suggesting that bases in these positions are important for RNA polymerase interaction. The sequences centered around -10 (TATTCT) and -35 (TTGACC) in this promoter are, in fact, prototypical of eubacterial promoters. Overlapping the RNA polymerase binding site is a perfect 12-bp direct repeat sequence. Some base changes within this direct repeat resulted in constitutive expression, suggesting that this sequence is an operator for negative regulation. Other base changes resulted in loss of glucose repression while retaining the requirement for chitin induction, suggesting that this sequence is also involved in glucose repression. The fact that cis-acting mutations resulted in glucose resistance but not inducer independence rules out the possibility that glucose repression acts exclusively by inducer exclusion. The fact that mutations that affect glucose repression and chitin induction fall within the same direct repeat sequence module suggests that the direct repeat sequence facilitates both chitin induction and glucose repression.

The regulation of carbon source utilization is an important aspect of the physiology of all microorganisms. The ability to recognize the presence of rapidly metabolizable carbon sources and regulate the synthesis of enzymes involved in the utilization of more complex carbohydrates requires the coordinated activation and repression of sets of genes. Although catabolite control of gene expression is common to most bacteria, the mechanisms employed to accomplish gene regulation at the molecular level are fundamentally different.

In *Streptomyces*, the regulation of carbon utilization is central to the most interesting and important aspects of its biology. It is clear from the study of mutants blocked in the initiation of development that there is a direct connection between carbon-source catabolite repression, activation of antibiotic biosynthesis, and morphological development, especially those aspects of morphological development that involve cell-cell signaling (1). Although relatively little is known about the mechanism of catabolite repression in *Streptomyces*, there are important differences in the regulation of carbon utiliza-

tion between *Streptomyces* and other bacteria. In contrast to *Escherichia coli* (2, 3), cAMP does not play a regulatory role in catabolite repression in *Streptomyces coelicolor*. Levels of cAMP do not change with changes in carbon source utilization in either *S. coelicolor* (4) or *Streptomyces venezuelae* (5), and null mutations in adenylate cyclase have no apparent effect on glucose repression (Charles Thompson, personal communication). In contrast to *Bacillus*, there is no detectable serine phosphorylation of HPr in *Streptomyces* (6, 7), so if the phosphotransferase system is involved in catabolite control in *Streptomyces* it may be by a novel mechanism. Instead, glucose kinase is required for the control of most (8–12) but not all (13) catabolite-controlled genes. Glucose repression of the *gyl* (11, 14), *dag* (15, 16), and *aml* (12, 17) promoters has been shown to be dependent on glucose kinase activity. Mutations in the glucose kinase gene of *S. coelicolor* result in the inability to utilize glucose as carbon source and in the loss of catabolite repression of some genes but have no effect on glucose transport (4). Furthermore, glucose kinase genes from other organisms or a normally cryptic glucose kinase gene of *S. coelicolor* are able to restore glucose utilization but not glucose repression to an *S. coelicolor glkA* mutant (9). Angell *et al.* (8) and Kwakman and Postma (18) have suggested that glucose kinase in *Streptomyces* may, in fact, function in a way that is similar to that of Hexokinase II in *Saccharomyces cerevisiae* (19–22). The involvement of glucose kinase in glucose repression has also recently been reported in *Staphylococcus xyloso* (23, 24). Taken together, these observations suggest that glucose kinase may be involved in a mechanism of catabolite control that exists in all Gram-positive bacteria.

Relatively few catabolite-controlled genes have been cloned and characterized from *Streptomyces*, and even fewer have been examined at the level of transcription initiation. The agarase gene, *dagA*, has four promoters (15) and is transcribed by at least three different RNA polymerase holoenzymes (16). The *galP1* promoter, which directs glucose-sensitive, galactose-dependent transcription of the *Streptomyces* galactose utilization operon, contains an unusual RNA polymerase binding site and is apparently transcribed by a new form of RNA polymerase holoenzyme (25). Mutational analysis of the promoter region of *galP1* also revealed a complex operator that consists of hexamer and direct repeat sequences that overlap the RNA polymerase binding site (26).

Of particular interest to this study is the analysis of the glycerol and maltose utilization operons of *S. coelicolor*. The *gylCABX* operon is transcribed from two promoters, both of which are glucose-sensitive and glycerol-dependent (11, 14, 27, 28) although to different extents (28). A repressor gene, *gylR*, has been identified and partially characterized (27–29). Hindle and Smith (29) showed, somewhat surprisingly, that null mutations in the *gylR* gene relieved glucose repression of both *gylR* and the *gylCABX* operon. Their data suggested that both substrate induction and glucose repression are mediated

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through the GylR protein. In recent work, Van Wezel *et al.* (30) have shown that transcription of the *malE* gene, which is part of the *malEFG* operon required for maltose utilization, is repressed by the product of the *malR* gene. Disruption or deletion of *malR* resulted in glucose-resistant expression of *malE*, indicating that this repressor plays a role in glucose repression as well as maltose induction. Both of these studies have relied on mutations in the repressor genes to suggest a role for the repressor proteins in glucose repression.

In this study we report an analysis of cis-acting mutations in an operator that define it as the site of glucose repression as well as substrate induction. Transcription from *chi63* is chitin-dependent and glucose-sensitive (13, 31, 32). In previous work we identified a transcription start site for this gene and showed that a direct repeat sequence upstream of the transcription start site is involved in regulation (13). A point mutation within the direct repeat sequence resulted in glucose-resistant, chitin-independent expression from *chi63 in vivo* (13). In electrophoretic gel mobility shift assays, we detected a protein in crude extracts from *S. coelicolor* that binds the direct repeat sequences specifically (13). In the analysis reported here, deletions of the promoter region were constructed, and a 350-bp region containing the transcription start site and the direct repeat sequence was shown to be sufficient for wild-type expression and regulation of *chi63*. The introduction of single base changes throughout the promoter region by random and site-directed mutagenesis identified an apparent RNA polymerase binding site prototypical of eubacterial promoters (TTGACC at -35 and TATTCT at -10) and a simple operator that apparently directs both glucose repression and chitin induction. We suggest that our work and that of Hindle and Smith (29) and Wezel *et al.* (30) provide evidence that glucose repression of some catabolite-controlled genes in *Streptomyces* may act through the same repressor proteins and operator sequences that are involved in substrate induction.

MATERIALS AND METHODS

Bacterial Strains. *Streptomyces lividans* 1326 (33) was used for analysis of wild-type and mutant *chi63* derivatives. RNA for primer extension analysis was prepared from *S. lividans* containing wild-type and mutant derivatives of the *chi63* promoter. *E. coli* strain DH5 α F' (34) was used for propagation of plasmids and M13 phage. *E. coli* BW313 (34) was used to prepare uracil-substituted single-stranded DNA for mutagenesis. Manipulations of *Streptomyces* (33) and *E. coli* were as described (34).

Construction of Deletion Derivatives of the *chi63* Promoter. A 850-bp *Hind*III-*Bam*HI fragment from pXE60 (13) containing the *chi63* promoter was cloned into M13mp18. Oligonucleotide-directed mutagenesis was used to engineer restriction sites at various positions within the promoter fragment to generate deletions. For upstream deletions with respect to the transcription start site, a *Hind*III site was introduced, and for downstream deletions a *Bam*HI site was introduced to allow directional subcloning of the promoter-containing fragments to generate transcriptional fusions to the *xylE* reporter gene. M13mp18 recombinant phages containing mutant derivatives of *chi63* were sequenced using the Sequenase kit from United States Biochemical to verify constructions.

Oligonucleotide-Directed Mutagenesis. A 560-bp *Hind*III-*Bam*HI fragment containing the *chi63* promoter was used as a substrate for mutagenesis. For random mutagenesis, two oligonucleotides were synthesized: a 37-bp fragment corresponding to the sequence from -50 to -14 and a 33-bp fragment corresponding to the sequence from -37 to -5 with respect to the transcription start site. Oligonucleotides were synthesized with 1.7% contamination of each of the other nucleotides at each position. For oligonucleotide-directed, site-specific mutagenesis, oligonucleotides were synthesized with specific base

changes. Incorporation of mutagenized oligonucleotides was performed according to the method of Kunkel and colleagues (35, 36). The pool of mutagenized promoter-containing fragments was subcloned into pXE3 upstream of the *xylE* reporter gene. Base changes that resulted in altered expression of *chi63* were detected using catechol dioxygenase plate assays.

Catechol Dioxygenase Assays. Both qualitative and quantitative catechol dioxygenase assays were performed as previously described (13, 37). For plate assays, *S. lividans* strains were grown in modified minimal medium containing either 1% glucose, chitin, or chitin plus glucose as sole carbon source for 48 hr at 30°C and then sprayed with a 1% (wt/vol) catechol solution. Plates were inspected within 5–10 min for color change. For quantitative assays, *S. lividans* strains were grown in minimal medium containing 1% glycerol for 28 hr at 30°C. Mycelia were collected by centrifugation, washed twice with 10 ml minimal medium containing no carbon source, then resuspended in 5 ml minimal medium, and aliquots were distributed into minimal medium containing 1% glycerol, glucose, chitin, or glucose plus chitin and incubated another 16 hr at 30°C.

Primer Extension Analysis. RNA for primer extension analysis was isolated from cultures grown as for catechol dioxygenase assays. RNA isolation from *Streptomyces* (33) for primer extension analysis (13) was done as previously described. The primer used for the extension reaction, 5'-GTCAGGCTAGGACCAGGTCC-3', was complementary to the 5' end of the *xylE*-containing fragment and did not hybridize with chromosomal DNA. Transcripts detected in this way were specific for RNA generated from the *chi63-xylE* fusion.

RESULTS

A 350-bp Region of the *chi63* Promoter Is Sufficient for Activity and Regulation. To identify the DNA region of *chi63* required for expression and regulation, a series of deletions 5' and 3' of the transcription start site was constructed (Fig. 1A). The modified promoter-containing fragments were cloned upstream of the *xylE* reporter gene, and quantitative catechol dioxygenase assays were performed (Fig. 1B). Deletions to +150 or -200 had no effect on expression or regulation of *chi63*. Deletions to +45 showed decreased expression but retained glucose repression and chitin induction. Deletions to +10 or -120 resulted in the complete loss of expression from *chi63*. The results of this analysis indicate that the DNA sequence from -200 to +150, with respect to the transcription start site, is sufficient for regulation and wild-type levels of expression from the promoter.

Uninducible Mutations Define Sequences Important for Promoter Activity. Single base changes that result in loss of promoter activity most likely identify bases important for RNA polymerase recognition and interaction. As shown in Fig. 2, a T-to-G transversion or T-to-C transition at -10, a T-to-G transversion at -12, an A-to-G transition at -32, a G-to-T transversion at -33, a T-to-G transversion at -35, or a T-to-G transversion at -37 resulted in complete loss of promoter activity. All of these changes are centered around -10 and -35 base pairs upstream of the transcription start site, positions where RNA polymerase would be expected to make contact.

Mutations Within a 12-bp Direct Repeat Sequence Affect both Chitin Induction and Glucose Repression. In previous work (13), a C-to-A transversion 18 bp upstream of the transcription start site and within the 12-bp direct repeat sequence resulted in glucose-resistant, chitin-independent expression, suggesting that the direct repeat sequences serve to bind a negative regulator of transcription in the way classical operators function to modulate repression. As shown in Fig. 2, base changes at -16, -18, -23, -24, -25, and -40 resulted in a constitutive phenotype. These mutations identify the direct repeat sequences as a site for negative regulation.

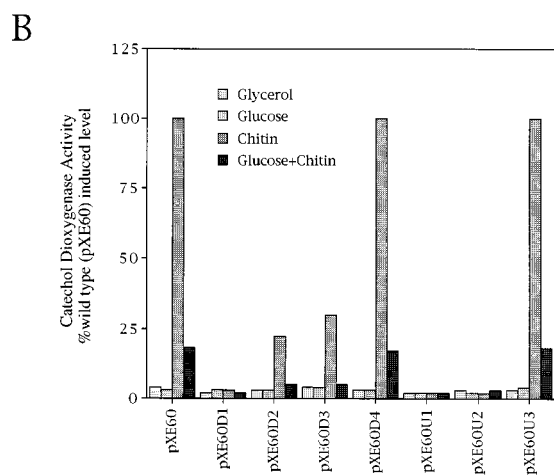
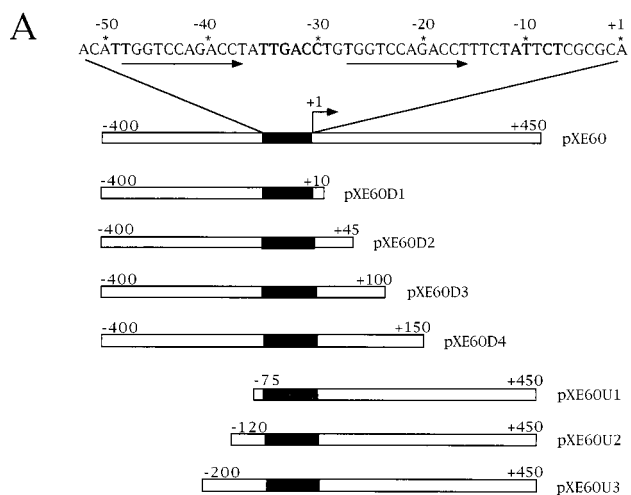


FIG. 1. Analysis of deletions of the *chi63* promoter region. (A) DNA sequence of the promoter region containing the RNA polymerase binding site (bold letters) and direct repeat sequences (underlined with arrows) and diagrams of deletions of an 850-bp region containing the transcription start site (indicated as +1). (B) Histogram showing the results of quantitative catechol dioxygenase assays plotted as a percentage of the fully induced wild-type level from *S. lividans*-containing plasmids bearing *chi63-xylE* transcriptional fusions. pXE60D1, pXE60D2, pXE60D3, pXE60D4, pXE60U1, pXE60U2, and pXE60U3 are pXE60 derivatives that contain different truncated versions of *chi63*. Promoter-containing plasmids indicated in B refer to the deletions shown in A. Each plasmid-containing strain was assayed after growth on the carbon source indicated.

Surprisingly, base changes at -19 and -21, also within the direct repeat sequences (Fig. 2), resulted in glucose-resistant expression but retained the requirement for chitin induction. These mutations did not affect the level of expression of the promoter. A T-to-G transversion at -16 also relieved glucose repression without affecting the requirement for chitin induction, but this change also had a dramatic effect on expression. The existence of these mutations suggests that the direct repeat sequences are also directly involved in glucose repression. The fact that mutations that give rise to these two different phenotypes are dispersed throughout the same direct repeat sequence suggests that the same sequences facilitate both chitin induction and glucose repression.

Of special interest is a base change located outside the direct repeat sequence and within the -10 region that relieves glucose repression. A T-to-A transversion at -9 resulted in glucose-resistant, chitin-dependent expression. Two additional base changes, a T-to-G transversion at -14, which resulted in

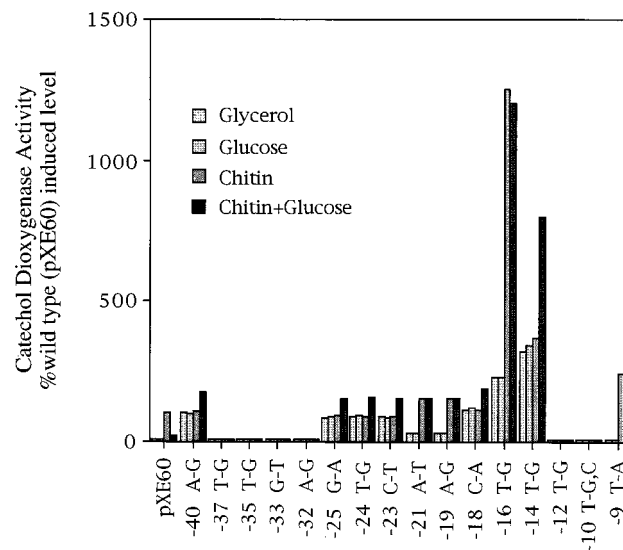


FIG. 2. Analysis of point mutations in the *chi63* promoter region. This histogram shows the results of catechol dioxygenase assays plotted as a percentage of fully induced wild type from *S. lividans* containing plasmids with *chi63-xylE* transcriptional fusions. Promoter mutations are labeled as to the position upstream of the transcription start site and the base change observed. Each plasmid-containing strain was assayed after growth on the carbon source indicated.

elevated expression, and a T-to-G transversion at -16, which affected expression and regulation, are located at the 3' end of the direct repeat sequence and immediately adjacent to or within the -10 region of the promoter. Their dual effect on regulation and promoter activity suggests the possibility of an interaction between RNA polymerase and the transcription factor that interacts with the direct repeat sequences.

Deregulated Expression from *chi63* Originates at the Same Transcription Start Site as That of the Wild Type. Because point mutations within the promoter region that affect regulation may also affect a variety of interactions, including RNA polymerase recognition and binding, it was important to establish the site of transcription initiation in these mutants. It was particularly important to examine the promoter mutations that resulted in dramatic overexpression, such as the changes at -16 or -14. As shown in Fig. 3, in all cases, transcription from the mutant *chi63* promoters originated from the wild-type start site. We conclude from these data that the base changes that affected regulation of *chi63* do not grossly alter the way in which RNA polymerase recognizes the promoter region to initiate transcription.

DISCUSSION

We report the isolation and characterization of base substitution mutations introduced throughout the *chi63* promoter region. Base changes located at -10, -12, -32, -33, -35, and -37 resulted in loss of promoter activity. The location of these mutations and the fact that they lie within sequences that are similar to those typical of eubacterial RNA polymerase recognition sequences, TATTCT at -10 and TTGACC at -35, suggest that bases in these positions are important for RNA polymerase interaction. There are at least five sigma factors in *Streptomyces* (38, 39) known to recognize these sequences or variations of these sequences. Overlapping the RNA polymerase binding site is a perfect 12-bp direct repeat sequence. Base changes within this direct repeat sequence at -16, -18, -23, -24, -25, and -40 resulted in glucose-resistant, chitin-independent expression. The constitutive phenotype of these mutations suggests that the direct repeat sequence is an operator that binds a negative regulator of transcription. Base

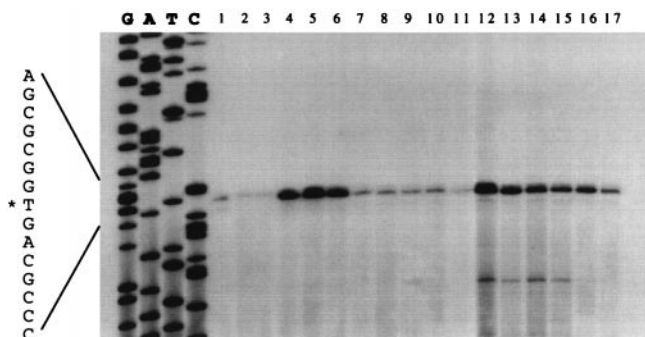


FIG. 3. Primer extension analysis of mutant *chi63*-directed transcripts. The 5' termini of transcripts were identified using a radiolabeled primer complementary to the RNA sequence 150 bp downstream of the transcription start site, extended with reverse transcriptase. RNA was isolated from *S. lividans* containing the wild-type *chi63* after growth on minimal media with chitin as carbon source (lane 1); with an A-to-G substitution at -40 , grown on glycerol (lane 2) or glucose (lane 3); with a T-to-G substitution at -14 , grown on glycerol (lane 4), glucose plus chitin (lane 5), or glucose (lane 6); with a G-to-A substitution at -25 , grown on glucose (lane 7); with a T-to-G substitution at -24 , grown on glucose (lane 8); with a C-to-T substitution at 23 , grown on glucose (lane 9); with a T-to-A substitution at -21 , grown on glucose (lane 10); with an A-to-G substitution at -19 , grown on glucose (lane 11); with a T-to-G substitution at -16 , grown on glucose (lane 12), glucose plus chitin (lane 13), or glycerol (lane 14); with a T-to-A substitution at -9 , grown on glucose plus chitin (lane 15); with a C-to-A substitution at -18 , grown on glucose plus chitin (lane 16); with an A-to-G substitution at -19 , grown on glucose plus chitin (lane 17). The same primer was used to prime dideoxynucleotide sequencing reactions from a single-stranded DNA template, M13mp19, containing the corresponding fragment of *chi63*. The letters above the sequence ladder indicate the dideoxynucleotide used to terminate each reaction. The asterisk indicates the nucleotide at the apparent transcription start site.

changes at -19 and -21 resulted in glucose-resistant, chitin-dependent expression, suggesting that the direct repeat sequence also facilitates glucose repression. The fact that mutations that give rise to these two different phenotypes are dispersed throughout the same direct repeat sequence module suggests that the same sequence facilitates both chitin induction and glucose repression.

Several lines of evidence suggest that the *chi63* repressor may mediate glucose repression as well as chitin induction. The fact that cis-acting mutations resulted in glucose resistance while retaining the requirement for chitin induction rules out the possibility that glucose repression acts exclusively by inducer exclusion. Because mutations outside the transcript coding region of *chi63* resulted in deregulated expression, regulation is at least in part at the level of transcription initiation. The existence of cis-acting mutations in the *chi63* operator that result in constitutive expression or relieve glucose repression indicate that the operator and, therefore, the repressor may mediate both chitin induction and glucose repression. The fact that some mutations lie in the putative RNA polymerase binding site indicated that regulation may involve the promoter, the repressor, and RNA polymerase acting in concert. These observations support and extend the work of Hindle and Smith (29) and Van Wezel *et al.* (30), who have shown that null mutations in the *gylR* and *malR* genes, which encode repressors of the glycerol (*gylCABX*) and maltose (*malE*) utilization operons, respectively, of *S. coelicolor*, relieve glucose repression of the entire operon. Their observations argue strongly that both substrate induction and catabolite repression are mediated through the *gylR* and *malR* proteins.

However, we have not rigorously ruled out the possibility that two different proteins, one that responds to chitin induction and another that responds to glucose repression, bind to overlapping targets within the direct repeat sequence. We also

have not ruled out the existence of a positive activator of transcription. The replacement of A-T base pairs with G-C base pairs at -9 , -14 , and -16 might be expected to reduce the melting efficiency of RNA polymerase during open complex formation. Somewhat surprisingly, these base changes resulted in a dramatic increase in expression from the promoter and may indicate the existence of a positive activator of transcription. In addition, the T-to-A substitution at -9 changes the -10 hexamer from TATTCT to TATACT, which is closer to the TATAAT consensus sequence. This could be interpreted to indicate that an activator that is required for promoter activity and is absent in the presence of glucose is not required in the -9 T-to-A mutant. This would be similar to the lacUV5 promoter, which functions independently of cAMP/CRP complex. It is also important to point out that bases upstream of the direct repeat sequences to -120 are required for full wild-type activity of the promoter and may include a binding site for such an activator.

Hindle and Smith suggested (29) a possible mechanism for catabolite repression of glycerol catabolism. They suggested that because glucose kinase is required for glucose repression of the *gyl* operon, the metabolism of glucose via glucose kinase may modulate the intracellular level of glycerol-3-phosphate, which might in turn determine the degree of repression by GylR. This model includes a role for glycerol kinase in regulating glycerol uptake and the possible involvement of the phosphotransferase system. Glucose repression of *chi63* transcription does not require glucose kinase, and only insoluble polymers of chitin act as inducer (refs. 31 and 32; Phillips Robbins, personal communication). Hindle and Smith (29) also showed that null mutations in GylR do not relieve glucose repression of agarase expression, suggesting that whatever mechanism is exerted through the repressor/operator may involve some specificity for given operators. For both *chi63* and *gylCABX*, evidence to date suggests that there is no general activator of transcription. We point out, however, that our analysis of *chi63* is not exhaustive and that our attempts to introduce random base changes throughout the promoter region were only partially successful. Furthermore, bases at positions shown to be important do not represent all possible changes. This is perhaps a limitation of the method of mutagenesis used to introduce base changes, because contaminated synthesis of oligonucleotides discriminates against the incorporation of some bases. The genetic and biochemical analysis of the regulation of operons such as *gylCABX*, which requires glucose kinase for glucose repression, and genes such as *chi63*, which does not, should contribute to our understanding of the mechanisms of catabolite control in this complex organism.

Several *Streptomyces* chitinase genes have been cloned and sequenced. *chi63* and *chi35* encode chitinases from *S. plicatus* (32). *chiC* and *chiA* encode chitinases from *S. lividans* (40), and *chi40* encodes a thermostable chitinase from *S. thermoviolaceus* (41). For *chi63*, *chi35*, *chiC*, and *chiA*, evidence that expression of these genes is glucose-sensitive and chitin-dependent has been reported. Transcription start sites have been identified for *chi63* (13), *chiA* (42), and *chiC* (40). *chi63* is the only gene for which cis-acting mutations define regulatory sequences. Although these chitinase genes are from different organisms and encode different enzyme activities, a comparison of the 5' regulatory regions of these genes revealed striking similarities. As shown in Fig. 4, each contains a putative RNA polymerase binding site prototypical of eubacteria and each contains a similar pair of direct repeat sequences. Interestingly, bases identified in our analysis as important for activity and regulation of *chi63* are highly conserved in all of these promoters. There are only two possible inconsistencies. The presence of a C in the third position of the putative -10 of *chi35* or an A in fourth position of the putative -10 region of *chi40* would not be consistent with our analysis. We point out that no transcription start site has been reported for either of these promoters and putative

chi63 CCACATTGGTCCAGACCTATTGACCTGTTGGTCCAGACCTTTC**TATTCTCGCGCCA**

chiC CCACATTGGTCCAGACCTATTGACCTATGGTCCAGACCTTTC**TATTCTCGCCGCA**

chi40 CGGTATTGGTCCAGACCCATTGACCCAGTGGTCCAGACCTTTC**TATATTTCGCGCCA**

chi35 CAAGAATGGTCCGAACCAATTGACCGCACCTGGTCTAGTCCCTC**TACGGT**GACGAA

chiA CCAACTTGGTCCGTACCTACCTC**TTGACAC**CTTCAATTGGTCTGGACC**CCCTTGTACGTCA**

FIG. 4. Comparison of the promoter regions of different chitinase genes. Underlined sequences indicate the direct repeat sequences. Bold letters indicate the putative RNA polymerase binding sites. Bold and underlined letters indicate the transcription start sites.

–10 region is deduced from sequence analysis. Also of interest is that in all cases, the direct repeat sequences in these promoters are positioned on the same face of the double helix. The spacing between the direct repeats is 19 bp in *chiA*, 9 bp in *chi63* and *chiC*, and 10 bp in *chi40* and *chi35*. It is also noteworthy that the direct repeat sequences lie in close proximity to or overlapping the RNA polymerase recognition sequences of these promoters. The existence of the same direct repeat sequence in the promoters of these genes suggests that they may be coordinately regulated by the same repressor and have similar mechanisms of transcriptional regulation. Because only insoluble polymers of chitin serve to induce *chi63*, it is possible that the inducer is sensed extracellularly and initiation of transcription of chitinase genes involves a signal transduction cascade.

The regulation of carbon utilization plays a central role in the most interesting aspects of the biology of *Streptomyces*. In response to nutrient depletion, including starvation for carbon, *Streptomyces coelicolor* initiates an elaborate process of morphogenesis and produces a wide variety of secondary metabolites, including four chemically distinct antibiotics (43, 44). Mutants blocked at the earliest stages of differentiation, known as *bld* (bald) mutants, are unable to erect aerial hyphae and are also defective to varying degrees in antibiotic production (45, 46). Interestingly, the morphological defects, and often the antibiotic deficiencies of most classes of *bld* mutants characterized to date, *bldA*, *bldC*, *bldD*, *bldG*, and *bldH*, are carbon-source-dependent. All of these mutant classes are defective in their ability to initiate differentiation and are blocked for antibiotic production when glucose is provided as the sole carbon source. When these mutants are grown on a poor carbon source such as mannitol, the ability to sporulate is partially restored, and, depending on the mutant class, so is the ability to produce antibiotics. We have recently shown that all classes of *bld* mutants [except for the very recently described *bldK* mutant (47), which has not been tested] are defective in the regulation of the *galP1* promoter and that the *bldB* mutant is globally deregulated for catabolite control (1). We have suggested that the *bld* genes may not be involved in morphogenesis *per se*, but instead play a central role in the ability of the organism to sense and/or signal starvation. The use of promoters such as *chi63* as tools may identify mutations and genes that elucidate the connection between the regulation of catabolite control and the initiation of morphogenesis.

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