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Catabolite Repression in the Gram-Positive Bacteria: Generation of Negative Regulators of Transcription

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Abstract Operons subject to catabolite repression (CR) in the gram-positive bacteria appear to be transcriptionally regulated by negative acting catabolite repressors. Cis elements within the promoter regions of a few CR operons have been identified as the target sequences for these repressors. It has also been proposed that sequences internal to the transcriptional unit may represent targets for recognition of the operons as catabolite repressible. The precise mechanism(s) of regulation have yet to be worked out. © 1993 Wiley-Liss, Inc.

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Many bacteria, when incubated in a growth medium containing glucose and additional carbon/energy sources, preferentially metabolize the glucose. The enzymes required for utilization of the alternate energy sources are not synthesized until the glucose has been exhausted from the medium [Neidhardt and Magasanik, 1956; Magasanik, 1961]. The effect is not restricted to glucose as other rapidly catabolized compounds, especially those transported by the phosphoenolpyruvate-dependent phosphotransferase system (PTS), are also effective [see Roseman and Meadow, 1990]. Catabolite repression is the general term to describe this type of regulation of carbon source preference. Catabolite repression is effected at the level of transcription, and the mechanisms have been well studied in the gram-negative bacterium *Escherichia coli.* The mechanism of regulation has two components. Catabolite repressible (CR) operons (lactose [*lac*], for example) are regulated in a "positive" sense by the binding of a complex of cyclic adenosine monophosphate (cAMP) and the cAMP receptor protein (CRP) dimer to a site in the promoter region of the operon designated CAP. This binding facilitates the recognition of the promoter by DNA-dependent RNA polymerase resulting in transcription of the structural genes of the operon. When glucose is transported into the cell, the $EIIA^{glc}$ component of the PTS (formerly termed $EIII^{glc}$, [Saier and Reizer, 1992]) inhibits the expression of the membrane-bound adenylate cyclase, lowering cAMP levels in the cell. The reduced cAMP levels result in loss of cAMP-CRP at the CAP site and consequent poor expression of the operon. The $EIIA^{glc}$ protein also inhibits the function of the LacY permease, resulting in the failure to transport lactose into the cell [Saier, 1989]. Reduction of cAMP and inducer exclusion effectively inhibit expression of the operon.

Catabolite repression has also been observed with gram-positive bacteria and has been best studied in *Bacillus subtilis, Streptomyces species,* and *Staphylococcus aureus.* Although the phenotype of catabolite repression with this group of bacteria is the same as that originally observed with *E. coli,* the mechanisms responsible are quite different. Many gram-positive bacteria, especially those with low DNA G+C content (30–40% range), lack detectable levels of cAMP [Botsford, 1992]. Others with high G+C content (70% range), such as *Streptomyces* species, possess cAMP but with no regulatory role for this compound determined [Demain et al., 1983]. Thus cAMP-independent mechanisms must be operative. Inducer exclusion and the more dramatic inducer expulsion [Reizer and Panos, 1980] have been described for the gram-positive group of bacteria. These processes would result in loss of the inducing signal within the

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TABLE 1. Cis Elements of Catabolite Repression in Gram-Positive Bacteria

Operon	Proposed cis element sequence	Positions[a]	Reference
S. aureus lac	TGTTTGTTTATG	−60 −25 (I)	Oskouian and Stewart, 1990
S. mutans lac	TATTTTTTAAA	−80 −55 (I)	Rosey and Stewart, 1992
B. subtilis citB	ATAAGT	−73 to −68 −64 to −59 (I) −32 to −27 (I)	Fouet and Sonenshein, 1990
B. subtilis amyE	TGTAAGCGTTAACA	−3 to +11	Weickert and Chambliss, 1990
B. subtilis gnt	ATTGAAAG	+137 to +148	Miwa and Fujita, 1990
B. subtilis xyl	34 bp sequence	+125 to +158	Jacob et al., 1991

[a]Nucleotide positions are given relative to the start site of transcription (+1). The values for *S. aureus* and *S. mutans* are approximations. (I) signifies the sequence is an inverted repeat of the given sequence.

cytoplasm of the cells and thus could explain the transcriptional repression observed. However, loss of inducing signal is insufficient to explain the transcriptional regulation seen in the gram-positives. Constitutive mutants, which do not require the presence of inducer for operon expression, still are transcriptionally repressible by addition of glucose to the culture. This has been demonstrated with the sucrose and histidine degradation operons of *B. subtilis* [Chasin and Magasanik, 1968; Lepesant et al., 1976] and the lactose operon of *S. aureus* [Oskouian and Stewart, 1987]. As no inducer is required for expression of these operons, the removal of inducer cannot explain the inhibition of transcription.

MECHANISM OF TRANSCRIPTIONAL REPRESSION ASSOCIATED WITH CATABOLITE REPRESSION

How is catabolite repression effected in gram-positive bacteria? Two models have been formulated. Both invoke negative regulators which bind to nucleic acid in the presence of a catabolite repression signal to prevent transcription of the CR operon. The first of these models, represented by the *B. subtilis* amylase (*amyE*) and aconitase (*citB*) and by the *S. aureus* and *S. mutans lac* operons [Fouet et al., 1990; Nicholson et al., 1987; Oskouian and Stewart, 1990; Rosey and Stewart, 1992], involve operator-like sequences within the promoter region of the CR operon. The target for the regulatory protein (the catabolite repressor) appears to be inverted repeat sequences. The arrangement in the *lac* and *citB* systems is similar (Table I). In *citB,* a 6 bp target sequence is positioned approximately 70 bp upstream of the transcriptional start site and is repeated twice more in an inverse orientation with the third element overlapping the −35 sequence of the promoter of the operon [Fouet and Sonenshein, 1990]. In *S. aureus lac,* the putative target sequence is 12 bp, is centered approximately 60 bp upstream of the transcriptional start point, and is perfectly repeated in an inverse orientation at a position between the −35 and −10 elements of the promoter of the operon [Oskouian and Stewart, 1990]. The *Streptococcus mutans lac* operon contains an 11 bp inverted repeat sequence with the repeats centered approximately 80 and 55 bp upstream of the transcriptional start site [E.L. Rosey and G.C. Stewart, 1992]. Deletion analyses have implicated the repeats as targets for the catabolite repressor [Fouet et al., 1990; Oskouian and Stewart, 1990]. The positions of the repeats suggest that DNA bending may be involved in the binding process.

The amylase (*amyE*) system in *B. subtilis* also entails an operator-like sequence [Nicholson et al., 1987; Weickert and Chambliss, 1989]. However, it is positioned downstream of the transcription start site (−3 to +11) rather than in the upstream promoter region and involves an imperfect repeat sequence. The operator-like site shares homology to the *E. coli lac* and *gal* operators [Nicholson et al., 1987]. The protein which binds to this target has been identified and its gene cloned [Henkin et al., 1991]. The protein is a 36.9 kDa polypeptide which shows similarity to the *E. coli* LacI (25% identity) and GalR (31% identity) repressor proteins. A consensus sequence for catabolite repression based on an analysis of mutations within the amyE sequence

has been postulated [Weickert and Chambliss, 1990]. A search of the DNA data base detected this sequence within several CR operons of *B. subtilis* [Weickert and Chambliss, 1990]. The positions of the putative cis elements relative to the transcriptional start site are variable.

A second model for transcriptional regulation in *B. subtilis* has been proposed [Miwa and Fujita, 1990]. Deletion analysis of the gluconate (*gnt*) operon has identified a cis element positioned within the first gene of the operon (Table I). A search for this sequence in other CR operons in *B. subtilis* has turned up identical or similar sequences, all positioned well downstream of the transcription start site [Miwa and Fujita, 1990]. These include the operons *hut* (ATTGAAAC, +201 to +208), *citB* (TTTAAAAG, +154 to +161), *sdh* (ATTGAAAG, +29 to +36), and *amyE* (TTTGAAG, +35 to +42). These sequences are the proposed binding sites for a negative regulator which may bind and bring about termination of transcription in an attenuator-like fashion [Miwa and Fujita, 1990]. Is ATTGAAAG a consensus sequence for catabolite repression in *B. subtilis* as proposed? Published data implicating this sequence exists only for the *gnt* operon. The other operons are implicated only through sequence gazing. Obviously, the conclusion for the *amyE* and *citB* operons are at odds with the role of the promoter region cis elements, unless two mechanisms operate independently in these operons. Interestingly, a downstream cis element has also been identified in the *xyl* operon of *B. subtilis* [Jacob et al., 1991]. However, deletion studies suggest a sequence homologous to the ATTGAAAG consensus (six out of eight matches) is not the critical cis element sequence. Furthermore, sequences matching the consensus sequence of Weickert and Chambliss as well as that of Miwa and Fujita are both present in the catabolite repressible *hut, sdh,* and *amyE* operons of *B. subtilis* [Miwa and Fujita, 1990; Weickert and Chambliss, 1990]. The role of these sequences in these operons remains to be experimentally determined.

Is there a global catabolite repression regulatory system in the gram-positive bacteria as exists for *E. coli?* The one known catabolite repressor, the CcpA protein of *B. subtilis* which acts on *amyE* has not yet been examined for possible effects on other CR operons. There are presently too few CR operons characterized from a single species to draw definitive conclusions. However, the variety of cis element sequences described above and also indirect evidence suggest that each operon may have its own specific catabolite repressor. When the *S. aureus lac* promoter fragment was introduced back into wild-type staphylococci on a multicopy plasmid vector (copy number approximately 10), the plasmid copies of the promoter region apparently titrated the catabolite repressor away from the chromosomal copy of *lac.* This rendered the plasmid-bearing cells unresponsive to regulation of *lac* by glucose [Oskouian and Stewart, 1990]. If the titrated catabolite repressor were a global regulator, it would of necessity be present in the cell at a higher concentration such that the limited number of copies of the plasmid-borne binding site would not have been expected to have such a pronounced effect. Thus it is likely that *lac,* and perhaps each CR operon, has its own catabolite repressor. The activation of the catabolite repressor when the cells are exposed to glucose in the environment may, however, involve a common, globally operative mechanism.

GENERATION OF THE SIGNAL TO ACTIVATE THE CATABOLITE REPRESSOR

Depending on the model, the putative catabolite repressor of a CR operon functions by binding to either the promoter region of the operon to prevent transcription by RNA polymerase or binds downstream of the transcriptional start site and results in termination of transcription. The resulting inhibition of transcription is only observed when the cells are grown in the presence of a carbon/energy source which invokes catabolite repression. The generation of a signal for catabolite repression could bring about the induction of synthesis of the catabolite repressor which would then bind to the cis element sequence and prevent transcription. Alternatively, the CR signal could result in activation of preexisting catabolite repressor. In this case, only the "activated" catabolite repressor would interact with the cis element. Both possibilities, induction of catabolite repressor synthesis or activation of the repressor itself, require a signal be generated to initiate the repression of the various operons. The signaling may require transmission to a variety of operon-specific catabolite repressors. At present, only the *B. subtilis* CcpA catabolite repressor has been identified and thus only in this system can these questions be specifically addressed.

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