

Genetic Dissection of the Light-Inducible *carQRS* Promoter Region of *Myxococcus xanthus*

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In *Myxococcus xanthus* photoprotective carotenoids are produced in response to illumination due to regulated expression of carotenoid biosynthesis genes at two loci. Induction of the carotenogenesis regulon is dependent on expression of the *carQRS* operon. The first gene product of the operon, CarQ, is a sigma factor belonging to the ECF family and is responsible for light-dependent initiation of transcription at the *carQRS* promoter. We defined the minimal *carQRS* promoter as a 145-bp fragment of DNA upstream of the *carQRS* transcriptional start site, which includes the promoter for a divergent gene, *gufA*. In order to elucidate regions with the promoter required for activity, point mutations were introduced into the *carQRS* promoter between positions –151 and 6. While most sequence changes abolished *carQRS* promoter activity, two changes enhanced promoter activity and two changes caused the mutant promoter to become constitutive and independent of CarQ. The promoter-null point mutations and 6-bp deletion mutations implied that the *carQRS* promoter requires a functional *gufA* promoter for transcriptional activity and vice versa. By mapping the extent of the promoter region, identifying sequences important for promoter activity, and highlighting potential topological effects, we provide a foundation for further analysis of the *carQRS* promoter.

Illuminating cells of the bacterium *Myxococcus xanthus* with blue light can cause lysis of the cells due to the light-dependent production of reactive species such as singlet oxygen and triplet-state protoporphyrin IX. As a survival response, *M. xanthus* produces a variety of photoprotective carotenoids by light-regulated expression of the *crt* biosynthetic genes (14, 29). Most carotenoid biosynthetic genes are located within the *crtEBDC* operon; the exception is the monocistronic *crtI* gene (1, 9). In the dark the *crtI* gene is transcriptionally silent, while the *crtEBDC* operon is repressed by CarA, which is encoded by the tenth open reading frame of the operon (40). Three regulatory factors governing carotenogenesis are encoded within the *carQRS* operon, which is expressed in a light-induced fashion (23). The first gene in the operon encodes an extracytoplasmic-function (ECF)-type sigma factor (18), CarQ, which directs transcription from the *crtI* and *carQRS* promoters (2, 23). The second gene product of the operon, CarR, acts as an anti-sigma factor (2, 11) by binding to CarQ and preventing CarQ-dependent initiation of transcription in the dark. The final polypeptide encoded in *carQRS* is CarS, which relieves repression of the *crtEBDC* operon by binding to the repressor CarA and eliminating its operator binding activity (40).

The ECF-type sigma factors (18) are a large class of sigma factors whose promoter recognition characteristics are poorly understood (12). The *carQRS* promoter possesses a –35 rec-

ognition hexamer which is very similar to the consensus ECF sigma factor-dependent promoter (Fig. 1). However, there is very little similarity to the consensus ECF-dependent promoter around nucleotide –10, a situation typical of ECF-dependent promoters (18). Transcription from the *carQRS* promoter requires expression of *ihfA* and *carD* (24, 27). IhfA is an alpha subunit of integration host factor, while CarD is a member of the HMGA family of DNA binding proteins. Binding sites for CarD have been found in the *carQRS* promoter region comprising a tandem repeat of the nucleotide sequence TTTCC (27). In addition to the requirement for *carQRS* transcription, CarD and IhfA are involved in other cellular processes. CarD is required for multicellular development (26), while IhfA appears to be necessary for normal rates of cell growth (24).

The promoters of *carQRS* and *crtI* are CarQ dependent, but an alignment of the transcriptional start sites revealed little obvious sequence similarity (Fig. 1). Additionally, in vitro experiments that showed CarQ-dependent transcription from the *carQRS* promoter failed to demonstrate transcription from the *crtI* promoter (2). A mutational analysis of the *crtI* promoter revealed critical nucleotide residues in blocks centered around nucleotide positions –31 and –10 (22). The minimal *crtI* promoter was found to start somewhere between nucleotides –54 and –25 and end somewhere between positions 57 and 120. A putative enhancer element was also identified between nucleotide positions 30 and 120 (22).

Two sigma factors belonging to the ECF subfamily other than CarQ have been identified in *M. xanthus*. RpoE1 (39) and RpoE2 (M. Ward, personal communication) are involved in regulation of motility behavior. As typically seen for promoters dependent on different ECF sigma factors, the promoters for *rpoE1* and *rpoE2* exhibit extensive sequence identity in the –35 region of the *carQRS* promoter but little identity around position –10 (Fig. 1). This suggests that differentiation between

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	-35	-10
<i>carQRS</i>	GCGCCGGAACACTTTTCGC-AGGT-GGCCCGTAGAGGAGTCG	
<i>ctrl</i>	GCTCTTGTAAACGTCCTGGC-GGTTTCGCGCGTTCCGCCAGGT	
<i>carQRS Mv</i>	C	G T CA
<i>rpoE1</i>	GCTAGGGAATATGTTCCCTGGCCGGCGTGGTATTATCCCGA	
<i>rpoE2</i>	ACTCGGGAAGGCATGTTTCTGGCTCAATATCTTGCAATTCA	
ECF consensus	CCGGAACCTT	TCT Y

FIG. 1. ECF sigma factor-dependent promoters of *M. xanthus*, including the consensus sequence for ECF sigma factor-dependent promoters (18). The *carQRS* *Mv* line indicates the sequence differences between the *carQRS* promoters of *M. xanthus* and *M. virescens*. The sequence of the *rpoE1* promoter is the sequence described by Ward et al. (39), and the *rpoE2* promoter sequence was kindly provided by Ward (personal communication). The -10 and -35 positions of the *carQRS* promoter are underlined, and known transcriptional start sites are indicated by boldface type. Bases are shaded if they are present in three of the four *M. xanthus* promoter sequences.

ECF sigma-dependent promoters by RNA polymerase holoenzymes is primarily determined by the promoter region around position -10. The lack of sequence conservation between the *carQRS* and other ECF-sigma factor-dependent promoters makes it necessary to dissect important regions within the *carQRS* promoter experimentally.

To determine the minimal promoter region of *carQRS*, we constructed a series of nested deletions of the *carQRS* promoter region using exonuclease III digestion. Once the minimal promoter was defined, we introduced a series of mutations throughout the *carQRS* promoter region and assayed the mutant promoters for light-induced transcription in *M. xanthus*. We also compared the sequence of the *carQRS* promoter region with that from another *Myxococcus* species, *Myxococcus virescens*.

MATERIALS AND METHODS

Bacterial strains and cultivation. The *Escherichia coli* strain used for all cloning procedures was MC1061 [*hsdR mcrB araD139 Δ(araABC-leu)7679 galU galK rpsL thi ΔlacX74(lacIPOZY)*], which was cultured as described by Sambrook et al. (32). The strains of *M. xanthus* used were DK101 (*sglA1*) (13) and UWM303 (*sglA1 carQ2*) (23). *M. virescens* Mxv4 was obtained from H. Reichenbach (GBF, Braunschweig, Germany). *M. xanthus* and *M. virescens* were cultivated as described by Hodgson (13).

Plasmids used. The plasmids used are described in Table 1. The derivation of plasmids is described below.

Oligonucleotide primer sequences. Oligonucleotide primers for PCR, mutagenesis, and sequencing were obtained from Gibco-BRL. The sequence of each primer used and the region in *carQRS* to which it anneals are shown in Table 2.

Transduction. For transfer of plasmids from *E. coli* into *M. xanthus*, coliphage P1 (*clr-100*) (31) was used according to the protocol described by Hodgson (13). To perform generalized transduction between strains of *M. xanthus*, myxophage Mx8 (21) was used as described by Hodgson (13).

DNA manipulation, nucleotide sequencing, and PCR. Restriction enzymes were used according to the manufacturers' specifications. DNA sequencing was performed by the method of Sanger et al. (33). PCR was performed according to standard protocols with *Taq* DNA polymerase. Reaction mixtures were supplemented with 10% dimethyl sulfoxide to overcome the high G+C content of the template DNA.

Construction of reporter plasmids. Plasmid pSJM101 was created by ligating a 5.6-kb PstI fragment containing Mx8 *attP* derived by partial digestion of pPR107 into pDAH274 that was also partially digested with PstI. Subsequent digestion with KpnI and religation yielded pSJM103, which possessed a single EcoRI site upstream of the promoterless *lacZ* gene. Plasmid pAEB130 was derived from pSJM103 by restriction with XbaI and ligation, which created unique BamHI and EcoRI sites upstream of the *lacZ* gene.

Mutagenesis of the *carQRS* promoter. To generate the Mut series of mutated promoters, plasmid pSJM100 was restricted with SmaI/MluI (nucleotide position 970 as described by McGowan et al. [23]) and ligated, which deleted 142 bp of DNA upstream from the minimal *carQRS* promoter, giving plasmid pAEB105. This plasmid was then digested with ClaI/XmnI (nucleotide position 1170 as described by McGowan et al. (23)) and ligated, which removed a 258-bp fragment carrying a truncated *carQ* gene, creating pAEB120. The 218-bp EcoRI fragment containing the *carQRS* promoter from pAEB120 was blunt ended and ligated into the SmaI site of pAlter1 (Promega Corporation), generating pAEB500. Mutagenesis of the promoter within pAEB500 was performed by using the Altered Sites II in vitro mutagenesis system (Promega Corporation) according to the manufacturer's instructions. Mutagenic primers were designed as described in the Altered Sites II in vitro mutagenesis system manual to generate the sequence changes shown in Fig. 2, which created novel KpnI restriction sites. Mutated promoters were removed from the pAEB500 plasmid backbone by digestion with EcoRI/BamHI and introduced into EcoRI/BamHI-restricted pAEB130. This generated plasmid pAEB600, which carried the wild-type *carQRS* promoter driving *lacZ* expression, and plasmids pAEB601 to pAEB616, each of which carried a promoter with a separate mutation (Table 1). Mutations were confirmed by DNA nucleotide sequencing and digestion with KpnI. To introduce 6-bp deletions into the *carQRS* promoter region, mutagenic primers (Table 2) were used in a PCR with plasmid pAEB120 as the template. Original plasmid DNA was removed by digestion with DpnI. The remaining DNA was then used to transform *E. coli*. For plasmids obtained in this fashion the presence of the mutation was confirmed by DNA nucleotide sequencing. The wild-type promoter from pAEB120 and mutagenized derivatives of this promoter were excised by digestion with EcoRI and ligated into EcoRI-digested pAEB130. The orientation of the wild-type and mutant promoters relative to the *lacZ* gene was determined by PCR. This procedure generated plasmids pSJB100 and pSJB101, which carried wild-type *carQRS* and *gufA* promoters, respectively, driving *lacZ* expression. Plasmids pSJB102 to pSJB109 carried mutant forms of the *carQRS* or *gufA* promoter driving *lacZ* production.

Insertion of promoter fragments into reporter plasmids. The 565-bp SacI/SmaI fragment of pDAH238 (*carQRS* region nucleotide positions 865 to 1430 as described by McGowan et al. [23]) was ligated into the SacI/SmaI sites of pIC19R to form pSJM100. Plasmid pSJM108 was created by inserting the 420-bp MluI fragment of pSJM100 (nucleotide positions 971 to 1391 as described by McGowan et al. [23]) into the MluI site of pMTL24, while the 249-bp Tacl fragment of pSJM100 (nucleotide positions 953 to 1202 as described by McGowan et al. [1993]) was ligated into pIC19R digested with ClaI to generate pSJM138. The EcoRI fragments of pSJM100, pSJM108, and pSJM138 containing *carQRS* promoter regions were ligated into EcoRI-digested pSJM103 to generate pSJM105, pSJM109, and pSJM139, respectively.

Exonuclease III deletion of promoter fragments. Plasmids pSJM100 and pSJM108 were used as the substrates for exonuclease III-mediated deletion. Digestion of pSJM100 with HindIII and PstI and digestion of pSJM108 with XhoI and SphI gave linear plasmids with 5' and 3' overhanging termini. Exonuclease III digests 3' to 5' along a DNA duplex with a 5' overhang, and its action on HindIII/PstI-digested pSJM100 and XhoI/SphI-digested pSJM108 resulted in deletion from the distal portions of the incorporated *carQRS* promoters. After digestion for various lengths of time, exonuclease III was removed by phenol extraction, and any overhanging termini were blunt ended with S1 nuclease and treated with the Klenow fragment of DNA polymerase I. Incubation with T4 DNA ligase resulted in plasmids which contained deleted promoters on EcoRI/EcoRI restriction fragments, which were subsequently introduced into the EcoRI site of pSJM103. Deleted promoters were sequenced and screened for appropriate orientation within the reporter plasmid.

Analysis of integration of reporter plasmids. The presence of reporter plasmids integrated into the chromosome was assessed by amplification by using primers CHECK1 and CHECK2 (Table 2) and standard protocols. Primer CHECK1 annealed within the *carQRS* promoter, while primer CHECK2 annealed to the coding strand within the *lacZ* gene. The PCR products obtained were screened by restriction enzyme digestion by using KpnI to confirm the presence of the Mut mutations. Introduction of plasmids into the *attB* locus occasionally leads to tandem insertions. Primers MULTI 1, MULTI 2, and MULTI 3 (Table 2) were used in PCRs to detect integrated reporter plasmids. Primer MULTI 1 was used at twice the normal primer concentration, along with normal quantities of both primer MULTI 2 and primer MULTI 3. A combination of primers MULTI 1 and MULTI 3 served as a positive control for any strain containing a portion of the *attP*^{Mx8} locus. Primers MULTI 1 and MULTI 2 gave a product if there was an intact *attP*^{Mx8} region, which was present only if upon integration into the chromosome multiple tandem copies of the plasmid inserted

TABLE 1. Plasmids used in this study

Plasmid	Genotype and/or phenotype ^a	Reference or source
pAEB105	<i>carQRS</i> promoter and truncated <i>carQ</i> MluI(position –165) to SmaI(position 294), Ap ^r	This study
pAEB120	<i>carQRS</i> promoter MluI (position –165) to ClaI (position 40), Ap ^r	This study
pAEB130	Promoterless <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pAEB500	<i>carQRS</i> promoter (positions –164 to 40), Tet ^r	This study
pAEB600	<i>carQRS</i> promoter (positions –164 to 40), <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pAEB601	Mut1 derivative of pAEB600	This study
pAEB602	Mut2 derivative of pAEB600	This study
pAEB603	Mut3 derivative of pAEB600	This study
pAEB604	Mut4 derivative of pAEB600	This study
pAEB606	Mut5 derivative of pAEB600	This study
pAEB607	Mut6 derivative of pAEB600	This study
pAEB608	Mut7 derivative of pAEB600	This study
pAEB609	Mut8 derivative of pAEB600	This study
pAEB610	Mut9 derivative of pAEB600	This study
pAEB611	Mut10 derivative of pAEB600	This study
pAEB613	Mut11 derivative of pAEB600	This study
pAEB614	Mut12 derivative of pAEB600	This study
pAEB615	Mut13 derivative of pAEB600	This study
pAEB616	Mut14 derivative of pAEB600	This study
pAlter1	<i>lacZ</i> α, Tet ^r	Promega Corporation
pDAH201	Mx DNA (HindIII 5.40L to BamHI 9.70R), Km ^r , +1/4IS50L, pMB <i>ori</i> , Ap ^r , P1inc	13
pDAH217	Mx DNA (HindIII 5.40L to BglII 0.37R), <i>lacZ</i> , P1inc, Ap ^r , p15A <i>ori</i>	13
pDAH238	Mx DNA (SmaI 0.79 L to SmaI 0.48R), Ap ^r , pUC <i>ori</i>	23
pDAH274	<i>lacZ</i> , P1incΔ, Ap ^r , p15A <i>ori</i>	34
pIC19R	<i>lacZ</i> , pUC <i>ori</i> , Ap ^r	20
pMTL24	<i>lacZ</i> , <i>par</i> , pUC <i>ori</i> , Ap ^r	4
pPR107	Mx8 <i>attP</i> , pUC <i>ori</i> , Ap ^r , <i>par</i>	30
pSJB100	<i>carQRS</i> promoter (positions –164 to 40) driving <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pSJB101	<i>gufA</i> promoter (positions –174 to 30) driving <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pSJB102	Δ <i>guf</i> derivative of pSJB100	This study
pSJB103	Δ <i>guf</i> derivative of pSJB101	This study
pSJB104	Δ <i>int</i> derivative of pSJB100	This study
pSJB105	Δ <i>int</i> derivative of pSJB101	This study
pSJB106	Δ <i>mid</i> derivative of pSJB100	This study
pSJB107	Δ <i>mid</i> derivative of pSJB101	This study
pSJB108	Δ <i>car</i> derivative of pSJB100	This study
pSJB109	Δ <i>car</i> derivative of pSJB101	This study
pSJM100	Mutant <i>carQRS</i> promoter and truncated <i>carQ</i> SacI(position –271) to SmaI(position 294), Ap ^r	This study
pSJM101	Promoterless <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pSJM103	Promoterless <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pSJM105	<i>carQRS</i> promoter SacI(position –271) to SmaI(position 294) driving <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pSJM108	<i>carQRS</i> promoter MluI(position –165) to MluI(position 255), Ap ^r	This study
pSJM109	<i>carQRS</i> promoter MluI(position –165) to MluI(position 255) driving <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study
pSJM138	<i>carQRS</i> promoter TaqI(position –183) to TaqI(position 66), Ap ^r	This study
pSJM139	<i>carQRS</i> promoter TaqI(position –183) to TaqI(position 66) driving <i>lacZ</i> , P1inc, Mx8 <i>attP</i> , Km ^r	This study

^a pUC *ori*, origin of replication from pUC; pMB *ori*, origin of replication from pMB; p15A *ori*, origin of replication from plasmid p15A; Tet^r, tetracycline resistance; Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Mx DNA (L to R), DNA from the *carQRS* locus of *M. xanthus* (the numbers in parentheses indicate positions as described by McGowan et al. [27]); +1/4IS50L, portion of IS50 of Tn5; P1inc, incompatibility locus from coliphage P1; P1incΔ, incompatibility locus from coliphage P1 deletion variant; Mx8 *attP*, *attP* locus from myxophage Mx8; *par*, pSC101 partition region.

(19). In no case were *M. xanthus* transductants found to carry multiply inserted reporter plasmids.

LacZ assays. In assays for the production of LacZ we utilized the chromogenic substrate *o*-nitrophenyl-β-D-galactose pyranoside and the method of Hodgson (13). The LacZ specific activities reported below are the averages of two or more independent assays.

Cloning and sequencing of the *M. virescens carQRS* promoter region. Chromosomal DNA was isolated from *M. virescens* Mxv4 as it was from *M. xanthus* by using the method of Gill et al. (10). DNA was amplified by PCR by using primers OrFX-2 and CarR2 (Table 2), and the products of two independent reactions were cloned into an M13 vector and sequenced. Comparison of the sequences of the two PCR products revealed only one ambiguity. Only unambiguous changes from the *M. xanthus* sequence are shown in Fig. 1 and 2C.

RESULTS

The minimal *carQRS* promoter requires 137 to 145 bp of DNA upstream of the transcriptional start site. To determine the extent of the minimal *carQRS* promoter, a reporter plas-

mid, pSJM103, was constructed to enable assays of the transcriptional activity in *M. xanthus* of any inserted DNA fragment (see Materials and Methods). Plasmid pSJM103 contains a promoterless *lacZ* gene downstream of a unique EcoRI restriction site for the insertion of *M. xanthus* DNA. This plasmid also carries the Mx8 *attP* region for integration into the *attB*^{Mx8} site in the *M. xanthus* chromosome (17). Reporter plasmids were integrated at the *attB*^{Mx8} locus rather than at the native locus to allow analysis of short (<200-bp) DNA regions and to prevent gene conversion events. Integration at the native locus by homologous recombination requires >700 bp of DNA and leads to tandem duplication of a mutant and a wild-type sequence. As the site of the crossover point is random, the mutant sequence need not be coupled to the *lacZ* gene. Also, gene conversion occurs at a very high frequency between tandem duplications in *M. xanthus* (35).

TABLE 2. Primer sequences^a

Primer	Sequence	Annealing site
Δguf _{for}	CACTAACGGCCTCG_AGGGCAGGACGGG	998
Δguf _{rev}	CCCGTCTGCCCCT_CGAGGCGCTTAGTG	998
Δint _{for}	CTGCTGGCGTTCG_CGTGACTTTCAG	1034
Δint _{rev}	CTGGAAAGTCACG_CGAACGCCAGCAG	1034
Δmid _{for}	GCTTTCTCTACCG_GAGAAGCGCGAGC	1066
Δmid _{rev}	GCTCGCGCTTCTC_CGGTAGAGGAAAGC	1066
Δcar _{for}	CCGGAACAC_AGGTGGCCCGTAGAGG	1099
Δcar _{rev}	CCTCTACGGGCACCT_GTGTTTCCGG	1099
CHECK1	GCGTCCGAGGTGCCTCCG	973
CHECK2	CGATCGGTGCGGGCCTCT	
MULTI 1	CGGACTCACGATGCTCGC	
MULTI 2	GACTCCTCTGGCTGGGTG	
MULTI 3	CAGCTTGGCACTCGGCAG	
CarR2	CCATCCGGGCCAGGAAGCCT	1239
ORFX-2	CTTCCGGCTGAGCTCCGAGG	856

^a The numbers indicate the first base of complementarity in the *gufA* *carQRS* region, according to the numbering scheme of McGowan et al. (23). CarR2 is the reverse primer and extends from position 1256 to position 1239 on the noncoding strand. Deleted bases represented by underlined spaces are present in the primers used to generate deletion mutations. CHECK2 anneals to the coding strand within the *lacZ* gene and was used with CHECK1 to ensure the presence of promoter probe sequences. MULTI 1, MULTI 2, and MULTI 3 anneal within the Mx8 *attB* region and were used to confirm single plasmid integration.

Three fragments of DNA that were defined lengths spanning the *carQRS* transcriptional start site were inserted into pSJM103. Plasmids pSJM105, pSJM139, and pSJM109 carried DNA from nucleotide positions –271 to 294, –183 to 66, and –165 to 255, respectively, where position 1 is the transcriptional start site (Fig. 2A). When pSJM103 was used to transduce a wild-type *M. xanthus* strain (DK101), LacZ production was low and light independent (~20 U of β-galactosidase/min/mg of protein) (Fig. 3), even though the bacteriophage fd major transcription terminator was upstream of the promoterless *lacZ* gene. After integration of pSJM105, pSJM139, and pSJM109 into the *M. xanthus* genome, assays for LacZ production revealed that each inserted fragment directed similar light-induced transcription of *lacZ* (Fig. 3). With the reporter constructs integrated at *attB*^{Mx8}, the levels of LacZ expression were low in the dark (~10 nmol/min/mg of protein); however, illumination (120 microeinsteins of white light/m²/s) resulted in greater levels of LacZ production (~60 nmol/min/mg of protein). Thus, each of the three DNA fragments assayed contained an intact light-inducible promoter, and consequently, the light-inducible *carQRS* promoter appears to lie between nucleotide positions –165 and 66.

When promoter probe pDAH217 was integrated by homologous recombination at the *carQRS* locus to form a merodiploid of the *carQRS* promoter region, the background levels of LacZ production were very low (2 nmol/min/mg of protein), and there was an induction maximum of around 200 nmol/min/mg of protein after 6 h of exposure to white light (13). Thus, integration at the Mx8 *attB* locus increased background *lacZ* expression about fivefold and reduced the level of light induction of the *carQRS* promoter about threefold compared to the promoter activity at the native locus. The behavior of the *carQRS* promoter integrated at *attB*^{Mx8} was significantly different from the activity at the native locus; however, the promoter activity remained light inducible and was consistent for several promoter probes (Fig. 3). Reduced activity of develop-

mental promoters located at Mx8 *attB* has also been observed previously (8, 17, 38).

By using pSJM100 (positions –271 to 294) and pSJM108 (positions –183 to 294) as progenitors, the *carQRS* promoter regions within each plasmid were serially deleted from the promoter distal end by using exonuclease III (see Materials and Methods). The deletants were sequenced, and a selection of deleted promoters were inserted into the EcoRI site of pSJM103. Plasmids carrying deleted *carQRS* promoter regions were introduced into *M. xanthus*, integrated into the *attB* locus, and then assayed for LacZ production. Two classes of LacZ production profiles were exhibited by the strains carrying deleted promoter fragments (data not shown). The first class behaved like plasmids pSJM105, pSJM109, and pSJM139 (i.e., four- to fivefold induction upon illumination). A second class of deleted fragments gave no light induction of *lacZ* expression. Fragments which contained at least 145 bp of untranscribed sequence permitted light-induced transcription (Fig. 2B). Conversely, the fragments containing DNA starting from position –136 or closer to the transcription start site exhibited no light induction of transcription (Fig. 2B). Thus, light-induced transcription from the *carQRS* promoter required between 137 and 145 bp of upstream untranscribed sequence. The large size of this promoter region suggested that accessory transcription factors and/or other components are required to elicit *carQRS* transcription.

Site-directed mutagenesis of the *carQRS* promoter region. In order to assess the activity of mutagenized promoters, reporter plasmid pSJM103 was digested with XbaI and religated so that it contained unique BamHI and EcoRI sites upstream of a promoterless *lacZ* gene (pAEB130). A region of DNA including the *carQRS* promoter (nucleotide positions –164 to 40 [Fig. 2A]) was used as the template for site-directed mutagenesis of the promoter region (see Materials and Methods). To identify regions within the promoter required for activity, engineered mutations were designed to span the promoter region, while unique KpnI sites were introduced. The mutant promoters are described in Fig. 2C and were designated Mut1 to Mut14. The mutant promoters were introduced into pAEB130 to generate the pAEB600 series of plasmids. The plasmids were used to transduce *M. xanthus*, and the presence of mutant promoters in kanamycin-resistant integrants was confirmed by PCR and restriction enzyme digestion (see Materials and Methods).

The wild-type promoter (positions –164 to 40) was also introduced into pAEB130 to generate pAEB600. After integration of pAEB600 into the *M. xanthus* *attB*^{Mx8} locus, LacZ production was assayed in both the light and the dark. The LacZ expression obtained was similar to that obtained with plasmids pSJM105, pSJM139, and pSJM109 (Fig. 3 and 4 and Table 3); i.e., there was moderate light induction (maximum activity, ~30 nmol/min/mg of protein), and there was significant background expression (~10 nmol/min/mg of protein) in the dark.

Mutations within the *carQRS* promoter region have diverse effects on activity. The effects of engineered mutations on the activity of the *carQRS* promoter are shown in Table 3. The induction profiles for wild-type, Mut1, Mut2, and Mut6 promoter regions are shown in Fig. 4.

The sequence changes introduced to create Mut1 (Fig. 2C)

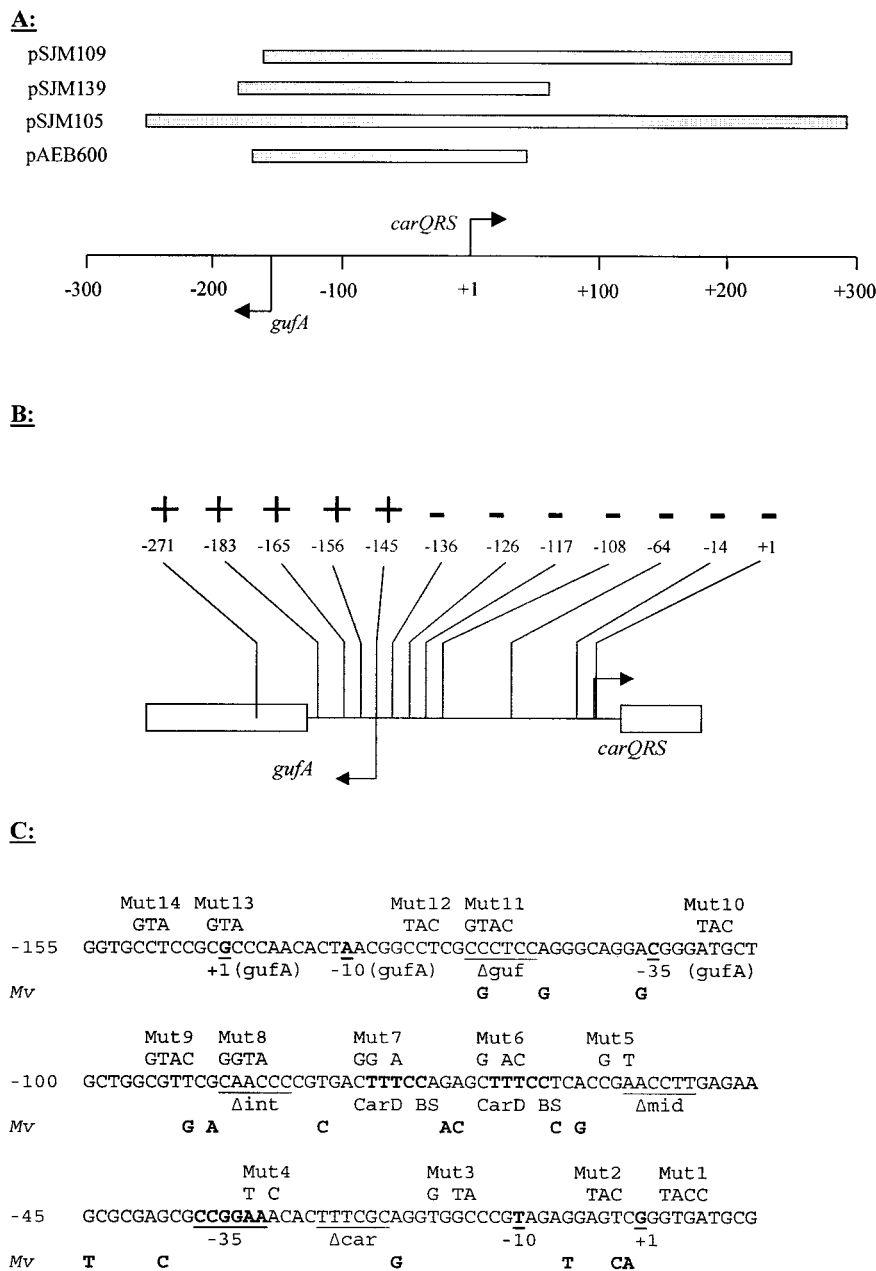


FIG. 2. *carQRS* promoter region and engineered mutations. (A) Extent of *carQRS* promoter regions inserted into reporter plasmids. The arrows indicate transcription start sites. (B) Deleted promoter fragments assayed for light-inducible promoter activity. The end points of exonuclease III digestion are indicated. In each case, the number refers to the nucleotide position of the deletion end point; a plus sign above a number indicates that the fragment was able to direct light-induced transcription, and a minus sign indicates that the fragment was not able to direct light-induced transcription. The arrows indicate transcriptional start sites, and the boxes indicate open reading frames of *gufA* and *carQRS*. (C) DNA sequence of the *carQRS* and *gufA* promoters. The proposed -35 recognition hexamer and -10 base of the *carQRS* promoter are underlined with boldface lines, as are the -35 and -10 bases of the *gufA* promoter and the transcriptional start sites. Mutagenized bases corresponding to each of the introduced mutations are indicated above the wild-type sequence. Bases deleted in 6-bp blocks are underlined and labeled under the wild-type sequence. The CarD binding sites (CarD BS) are in boldface type. Below the *M. xanthus carQRS* promoter sequence, bases which are different in the *carQRS* promoter of *M. virescens* are shown (lines labeled *Mv*).

resulted in a complete loss of light induction, although significant light-independent transcriptional activity (~ 10 nmol/min/mg of protein) was retained (Fig. 4 and Table 3). A requirement for particular bases in the transcribed, untranslated leader region of some genes has been observed in several systems (3, 15, 16).

The Mut2 alterations (Fig. 2C) had no significant effect on promoter activity, and illumination increased *lacZ* expression by 20 nmol/min/mg of protein (Fig. 4 and Table 3). It seems that the nucleotide sequence of the promoter region around nucleotide -3 was not critical for promoter activity.

The mutation in promoter Mut3 was close to the predicted

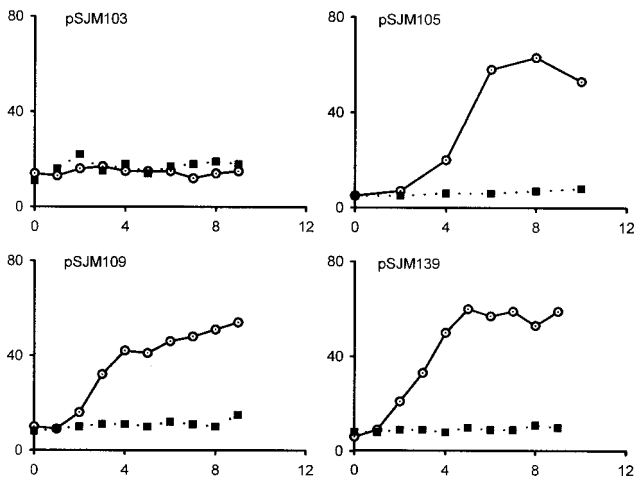


FIG. 3. Light induction of *carQRS* promoter fragments fused to *lacZ* and integrated at *attB^{Mx8}*. Each panel is a plot of the specific activity of β -galactosidase (in nanomoles per minute per milligram of protein) versus time (in hours). The reporter plasmid pSJM103 contained a promoterless *lacZ* gene. Plasmids pSJM105, pSJM109, and pSJM139 contained fragments of DNA spanning the *carQRS* promoter region (Fig. 2A). Plasmids were integrated into the *attB^{Mx8}* sites of wild-type *M. xanthus*, and the resulting strains were assayed for LacZ expression both in the light (circles) and in the dark (squares). Assays were performed at least in triplicate, and representative data are shown.

–10 recognition region of the *carQRS* promoter (Fig. 2C). After introduction into wild-type *M. xanthus*, Mut3 directed light-independent production of high levels of LacZ (~1,950 nmol/min/mg of protein [Table 3]). The sequence change in Mut3 presumably enabled the *carQRS* promoter region to be recognized by a sigma factor other than CarQ (σ^X). The Mut3:*lacZ* fusion was therefore used to transduce UWM303 (a strain lacking an intact *carQ* gene) by Mx8-mediated gen-

TABLE 3. Activities of mutant promoters ^a			
Mutation	Sp act in the dark	Peak sp act during induction	Induction factor
Wild type	10	30	3.0
Mut1	5	6	
Mut2	20	39	2.0
Mut3	1,440	1,950	High, constitutive
Mut4	43	48	Mid, constitutive
Mut5	18	68	3.8
Mut6	8	62	7.8
Mut7	2	3	
Mut8	1	2	
Mut9	2	2	
Mut10	1	1	
Mut11	2	3	
Mut12	1	2	
Mut13	11	11	
Mut14	13	16	

^a The promoter activities are expressed as specific activities of β -galactosidase (in nanomoles per minute per milligram of protein). The promoter activities shown are the maximum levels of β -galactosidase expression during induction and the mean levels of β -galactosidase expression in cells maintained in the dark for 12 h. The degrees of induction of promoter activity upon illumination are also shown for light-induced promoters. Assays were performed at least in triplicate, and representative data are shown.

eralized transduction (see Materials and Methods). In this mutant background, LacZ expression by Mut3 was even higher than the expression in the wild-type background (>25,000 nmol/min/mg of protein), confirming that Mut3 expression was CarQ independent and suggesting that in the wild type, CarQ RNA polymerase holoenzyme ($\alpha_2\beta\beta'\sigma^{\text{CarQ}}$) actively hinders expression of the Mut3 *carQRS* promoter, possibly by occluding binding by the σ^X RNA polymerase holoenzyme ($\alpha_2\beta\beta'\sigma^X$).

The Mut4 sequence changes coincided with the proposed –35 region of the promoter (Fig. 2C) and removed light-induced promoter activity, while they raised the background transcriptional activity to about threefold that of the no-promoter-region insert control (pSJM103) (Table 3 and Fig. 3). The loss of light induction implies that the Mut4 promoter interacted with $\alpha_2\beta\beta'\sigma^{\text{CarQ}}$ less efficiently.

Mutant promoter regions Mut5, Mut6, and Mut7 (Fig. 2C) differed from the wild-type sequence in the vicinity of the CarD binding sites (27). Both Mut5 and Mut6 promoters were induced by light to higher levels than the wild-type promoter (Fig. 4 and Table 3). Conversely, the Mut7 promoter showed an almost complete loss of activity, including the background expression level (Table 3). It was confirmed by PCR that the increased activity of the Mut5 and Mut6 promoters was not due to multiple insertions of plasmid DNA (see Materials and Methods). The mutations in promoters Mut6 and Mut7 were at the two proposed CarD binding sites identified by Nicolas et al. (27), yet the mutations had different effects on promoter activity, which might suggest that the two CarD binding sites have different functions within the promoter.

Sequences essential for activity are present between positions –74 and –155 of the *carQRS* promoter region. All mutations engineered between nucleotides –86 and –151 (Mut7 to Mut14) (Fig. 2C) essentially abolished light-induced transcriptional activity (Table 3). Most of the mutations in this region (Mut7 to Mut12) also resulted in a reduced level of background transcriptional activity. The loss of promoter activity caused by these mutations implies that the region be-

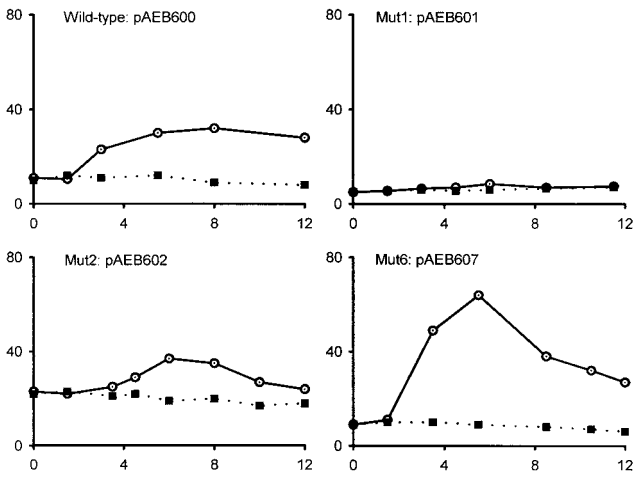


FIG. 4. Light induction of mutated *carQRS* promoters. Each panel is a plot of the specific activity of β -galactosidase (in nanomoles per minute per milligram of protein) versus time (in hours). Plasmids containing the *carQRS* promoters (wild type, Mut1, Mut2, and Mut6) were integrated into the *attB^{Mx8}* sites of wild-type *M. xanthus*, and the resulting strains were assayed for LacZ expression both in the light (circles) and in the dark (squares). Assays were performed at least in triplicate, and representative data are shown.

tween nucleotides –86 and –151 is critical for activity and broadly supports our observation concerning the size of the minimal promoter. Intriguingly, the extent of the *carQRS* minimal promoter region corresponds approximately to the start of the divergent *gufA* promoter (Fig. 2C). It is interesting that although deletion of the promoter region to nucleotide –145 allowed light induction, the sequence changes in Mut14 abolished promoter activity. Presumably, in the nucleotide –145 deletion construct other sequences were able to compensate and allow transcription.

The fact that most sequence changes led to alterations in promoter activity raised the possibility that most of the 150-bp sequence of the promoter region was constrained. *M. virescens* is closely related to *M. xanthus* (28). Southern blot analysis revealed the presence of *carQRS* homologues (data not shown), and carotenogenesis is light induced. We obtained the *carQRS* promoter region by PCR using primers derived from the *M. xanthus carQ* and *gufA* genes (see Materials and Methods). The sequences of the PCR products revealed 16 changes in the promoter region (Fig. 1 and 2C). There were five changes in the region from position –39 to position 1 (Fig. 1). Only three of these changes coincided with regions altered in the Mut1 to Mut14 mutations (i.e., Mut2, Mut9, and Mut11) (Fig. 2C). As indicated above, Mut2 had no effect on *carQRS* promoter activity. Only one base of the four bases changed in Mut9 and Mut11 was altered in *M. virescens*. Thus, if the *carQRS* region of *M. virescens* is CarQ dependent and light inducible, there are sequence changes that can be made while light inducibility is retained.

CarQ interaction with mutant promoters. Mutant promoters Mut4 and Mut1 both lacked light-induced transcriptional activity. However, the transcriptional activity of Mut4 in the dark was greatly elevated compared to that of the wild-type and Mut1 promoters. When mutant promoter Mut3 was introduced into UWM303, a strain with *carQ* deleted, the transcriptional activity of the Mut3 promoter increased by more than an order of magnitude, implying that $\alpha_2\beta\beta'\sigma^{\text{CarQ}}$ interfered with $\alpha_2\beta\beta'\sigma^{\text{X}}$ -dependent transcription. To characterize the difference between expression of the Mut1 promoter and expression of the Mut4 promoter, the wild-type, Mut1, and Mut4 *carQRS* promoters were introduced into UWM303, and their transcriptional activities were assessed.

All three promoters exhibited increased, light-independent activity in UWM303 compared to the activity in DK101 (wild type) (Table 3). The specific activity of the wild-type promoter (58.0 ± 0.5 nmol/min/mg of protein) was almost sixfold higher than the specific activity seen in DK101. The UWM303 Mut4 promoter activity (58.1 ± 1.1 nmol/min/mg of protein) was similar to the activity of the wild-type promoter and represented a 35% increase over the activity seen in DK101. The UWM303 Mut1 promoter activity (23.5 ± 0.8 nmol/min/mg of protein) represented about a fivefold increase over the DK101 activity. The presence of functional CarQ appears to reduce the transcriptional activity of the *carQRS* promoter even in the dark, when most CarQ would be expected to be complexed with CarR and therefore inactive. In a CarQ[–] background, the reduced activity of the Mut1 promoter compared to the wild-type and Mut4 promoters may imply that the Mut1 sequence changes affect transcription efficiency, independent of the sigma factor involved.

Transcriptional coupling of the *gufA* and *carQRS* promoters.

The upstream portion of the *carQRS* promoter region may include the binding site(s) for one or more required transcription factors. Alternatively, it is possible that this region is critical for activity because of a requirement for transcription of the *gufA* promoter, a situation seen in several divergent promoter systems (6). The *gufA* promoter is constitutively active throughout growth (β -galactosidase specific activity, 150.3 ± 16.4 nmol/min/mg of protein), but it is mildly light inducible (β -galactosidase specific activity, 187.5 ± 9.1 nmol/min/mg of protein) when it is measured by using *lacZ* transcriptional fusions at the *gufA carQRS* locus. If *carQRS* promoter activity requires a topological condition provided only during transcription of *gufA* (e.g., elevated levels of negative supercoiling), then mutation of the *gufA* promoter would abolish *carQRS* promoter activity. In order to discriminate between these two possibilities, we generated a set of mutations in the *carQRS* promoter region that each deleted six nucleotides (i.e., one-half a DNA helical turn). It might be expected that such a deletion would affect the *carQRS* and *gufA* promoters differentially if the upstream region contained a transcription factor binding site that influenced just one of the promoters but would affect the two promoters similarly if each promoter depended on transcription from the other.

Plasmid pAEB120 carrying the *carQRS* promoter was used as a template for mutagenesis (see Materials and Methods), and four separate deletions were introduced (Fig. 2C). Deletion Δguf was situated between the –10 and –35 regions of the *gufA* promoter, while Δcar was between the –10 and –35 regions of the *carQRS* promoter. Deletion Δmid was located between the *carQRS* promoter –35 region and the CarD binding sites, while deletion Δint was between the CarD binding sites and the –35 region of the *gufA* promoter (Fig. 2C). Mutated promoters were excised from pAEB120 by using EcoRI and were inserted into the EcoRI site of plasmid pAEB130. Consequently, plasmids which contained the promoter region in both orientations were obtained, which allowed in vivo assays of both *carQRS* and *gufA* promoter activities. Plasmids carrying wild-type and mutant promoters were introduced into the *attB*^{Mx8} locus and assayed for LacZ production.

The wild-type *gufA* promoter showed constitutive activity at the *attB*^{Mx8} locus (although the activity was lower than that at the native locus), and the wild-type *carQRS* promoter was light induced (Fig. 5). The Δint promoter mutant exhibited wild-type activity for both the *gufA* and *carQRS* promoters (Fig. 5). This implied that the site of the Δint deletion was between the *carQRS* and *gufA* promoters and that reorienting one promoter with respect to the other did not affect the activity of either promoter. In contrast, for the Δmid , Δguf , and Δcar promoter mutants, the *carQRS* promoter activities were not light inducible and *gufA* promoter activities were abolished or severely attenuated (Fig. 5). It seems that the *gufA* promoter requires an active *carQRS* promoter for activity and vice versa. Since disruption of one promoter disabled both promoters, it is possible that the large size of the minimal *carQRS* promoter actually reflected the location of essential sequence elements within the *gufA* promoter, which were also thus essential for the *carQRS* promoter.

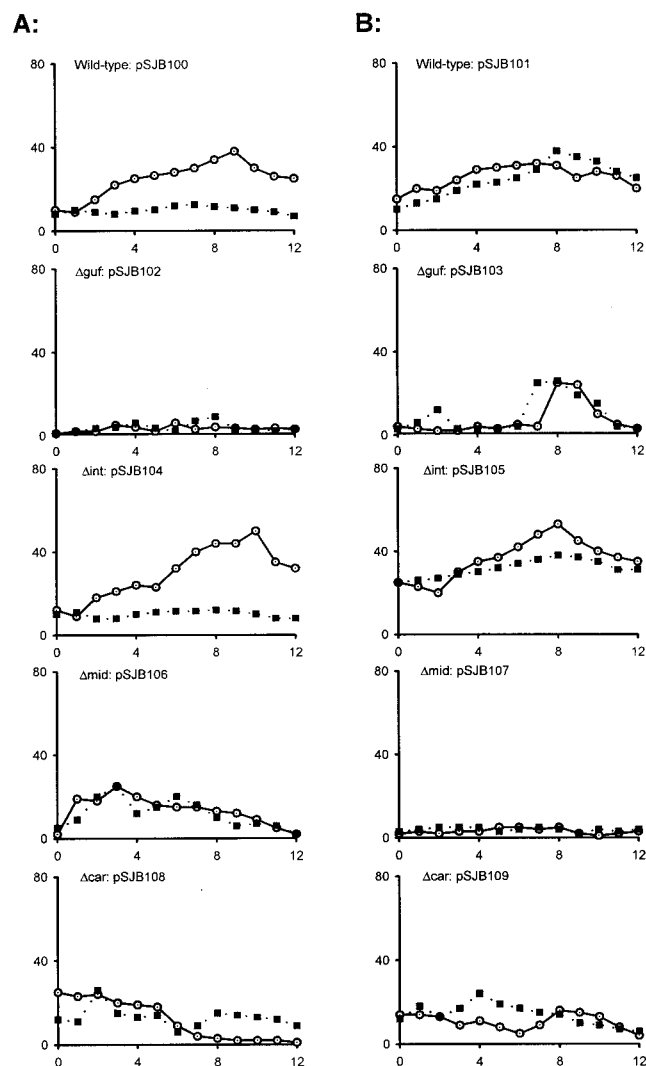


FIG. 5. Activities of *carQRS* and *gnfA* promoters carrying 6-bp deletions. Each panel is a plot of the specific activity of β -galactosidase (in nanomoles per minute per milligram of protein) versus time (in hours). Plasmids carrying promoters were integrated into the *attB*^{Mx8} sites of wild-type *M. xanthus*, and the resulting strains were assayed for LacZ expression both in the light (circles) and in the dark (squares). (A) Activity of wild-type and mutant *carQRS* promoters. (B) Activity of wild-type and mutant *gnfA* promoters. Assays were performed at least in triplicate, and representative data are shown.

DISCUSSION

Transcription of *carQRS* requires around 150 bp of DNA upstream of the transcription start site. The minimal DNA fragment required for light-dependent transcription of the *carQRS* operon consists of around 150 bp upstream of the transcriptional start point. DNA fragments that were different sizes and spanned the *carQRS* transcriptional start site were introduced into a reporter gene system and assayed for in vivo activity. The pattern of activity revealed that the minimal light-inducible promoter was somewhere between nucleotides -145 and 40 . Expression of LacZ in the light was reduced about twofold in DK101::pAEB600 compared to DK101::pSJM105, DK101::pSJM109, and DK101::pSJM139 (Fig. 3 and 4). This might imply that DNA between positions 40 and 66 changes

the mRNA stability or has enhancer activity, as seen for *crtI*. In the case of the *crtI* promoter, the early transcribed region has been shown to enhance transcription through active stimulation of transcription rather than stabilization of the nascent RNA (22). Mutations introduced into the *carQRS* promoter at positions -151 and -149 (Mut14) and positions 3 and 6 (Mut1) abolished light-induced promoter activity in vivo, supporting the broad definition of the minimal promoter region.

Disparate regions of the *carQRS* promoter affect promoter activity. Mutations engineered to span the minimal promoter region resulted in a wide variety of changes in promoter activity. Only one mutation (Mut2) failed to affect the light induction of promoter activity. In contrast, the comparison between the *M. virescens* and *M. xanthus carQRS* promoter regions revealed that there are at least 16 changes that can be made in the sequence and yet not eliminate light-inducible promoter activity. Two mutations (Mut5 and Mut6) enhanced the strength of light-dependent transcription initiation in vivo. The Mut2 mutation is between the -10 region and the transcription initiation site and thus might not be expected to be critical for promoter activity. The Mut5 and Mut6 promoter sequence changes are upstream of the -35 recognition region and, in the case of Mut6, overlap a proposed binding site for the CarD transcriptional activator. The Mut7 promoter has sequence changes that overlap the second CarD binding site, yet it renders the promoter completely inactive; thus, it appears that the two CarD binding sites in the *carQRS* promoter might have different roles. The positions of the Mut5, Mut6, and Mut7 sequence changes relative to the -35 promoter element hint that there is interference with the normal binding of CarD and/or possibly IhfA.

Sigma factor exchange mutation. A particularly dramatic change in promoter activity was seen with the Mut3 promoter, where transcription was enhanced by several orders of magnitude and rendered light independent. We proposed that the increase in transcription was due to recognition of the mutant promoter by an RNA polymerase holoenzyme with a sigma factor other than CarQ, σ^X , which was constitutively active. This hypothesis was proven by the observation that transcription from Mut3 was possible in a *carQ* mutant. In fact, transcription from the Mut3 promoter was greater in the *carQ* deletion mutant, which implied that CarQ could still recognize the Mut3 promoter enough to interfere with $\alpha\beta\beta'\sigma^X$ -dependent transcription. Surprisingly, this interference was also apparent in the wild-type strain in the dark, conditions under which CarQ is believed to be kept in an inactive state by CarR (2). Whether σ^X directed transcription from the *carQRS* transcriptional start site or was able to recognize a new promoter within the Mut3 construct is unknown.

Importance of the sequence of the *carQRS* mRNA leader. The Mut1 mutant promoter exhibited no light-induced transcription. The nucleotide bases altered in the Mut1 mutation are in the untranslated leader region of the *carQRS* mRNA. It has been noted that the base composition of the leader region can have effects on transcriptional activity (3, 15, 16), and an example is provided by the *crtI* promoter (22). It is possible that the Mut1 mutation affects RNA stability, RNA polymerase core pausing, or $\alpha\beta\beta'\sigma^{\text{CarQ}}$ initiation. Our data do not distinguish among these possibilities.

CarQ interaction with mutant promoters. On the basis of a comparison of the background promoter activities at the *attB*^{Mx8} site in mutant *carQ* and *carQ*⁺ strains (Table 3), it was clear that the presence of a functional copy of *carQ* inhibited expression from the wild-type promoter (6-fold decrease), the Mut1 promoter (5-fold decrease), and the Mut3 promoter (more than 13-fold decrease), while the Mut4 promoter was less affected (25% decrease). These observations suggest that Mut4 is defective for an interaction with CarQ, while the Mut1 and Mut3 promoters retain CarQ binding activity, although they do not necessarily retain CarQ-dependent transcriptional activity. Inhibition of transcription by CarQ was also apparent in the dark, which implies that the low level of CarQ present in the dark has a significant role.

***gufA* and *carQRS* promoters appear to be transcriptionally coupled.** None of the *carQRS* promoter mutations upstream of the CarD binding sites showed significant light induction of transcriptional activity, and the background activities with Mut7 to Mut12 were greatly reduced. The large size of the minimal *carQRS* promoter might reflect the presence of the binding site(s) for a necessary transcription factor(s) that operates through a DNA looping mechanism. In most cases of activation through DNA looping, the loop is stabilized by architectural DNA binding proteins that induce or stabilize bends in the DNA double helix. Relevant examples include integration host factor (37) and members of the HMGA family of proteins (36). In *M. xanthus* both integration host factor (24) and an HMGA protein, CarD (26), have been shown to be required for transcription of *carQRS* in vivo, although neither was required in an in vitro assay (2). While binding sites for CarD within the *carQRS* promoter have been identified (27), a binding site(s) for IhfA has yet to be identified.

If a promoter exists on a stretch of DNA that is topologically constrained, transcription from the promoter results in accumulation of positive supercoils downstream of the transcription complex and negative supercoils upstream of the transcription machinery. Increased levels of negative supercoiling favor open complex formation during transcription initiation. Therefore, if two divergent promoters are found within a single topologically constrained domain, they each stimulate transcription of the other promoter (that is, they are transcriptionally coupled) (5, 6). If such a topological domain does exist at *carQRS*, one might predict that disruption of the *gufA* promoter would affect the activity of both of the *gufA* and *carQRS* promoters. In this case, the presence of an essential upstream region within the *carQRS* promoter might merely reflect the requirement for an intact *gufA* promoter for *carQRS* transcription.

In order to discriminate between the alternative models of transcription factor binding and transcriptional coupling, a series of mutant promoters which had deletions of 6-bp stretches were constructed. Since there are approximately 11 bp per helical turn of double-stranded DNA, removal of 6 bp results in DNA on one side of the deletion that is rotated ~180° relative to the DNA on the other side of the deletion. The effects of all four 6-bp deletions on the activities of both the *carQRS* and *gufA* promoters were assessed in vivo.

The Δint mutation was a 6-bp deletion between the CarD binding sites and the *gufA* promoter (Fig. 2). This change had no significant effect on *carQRS* or *gufA* promoter activity (Fig.

5). If a transcription factor binding site required for *carQRS* promoter activity was to be found upstream of the deleted bases, then the reorientation of the binding site relative to the promoter would be expected to abolish promoter function. Support for transcriptional coupling between the *carQRS* and *gufA* promoters was provided by the effects of the Δguf and Δcar mutations. Six-base-pair deletions were engineered between the -35 and -10 regions of the *gufA* (Δguf) and *carQRS* (Δcar) promoters. We assumed that such deletions would render the corresponding promoters inactive, since precise separation of these promoter elements is typically critical for promoter function. When the in vivo activities of the mutants were assessed, it was found that in both mutants transcription of *gufA* had been abolished and the activity of the *carQRS* promoter was no longer light induced. Thus, it appears that it is impossible to inactivate one promoter without disabling the other promoter, implying that there is transcriptional coupling between the two promoters. Transcriptional coupling was also observed for the Δmid mutation, in which 6 bp was deleted between the CarD binding sites and the -35 region of the *carQRS* promoter. As described above, the mutation abolished transcription of *gufA* and *carQRS*. Since the Δmid mutation abolished *carQRS* promoter activity yet the Δint mutation did not, the orientation of the nucleotide sequence between positions -50 and -83 appears to be vital for *carQRS* transcription, suggesting that there is a transcription factor binding site. Indeed, this region of the *carQRS* promoter does contain the two CarD binding sites.

As the loss of *gufA* promoter activity causes loss of light-inducible *carQRS* promoter activity, the implication is that Mut14 is another mutation like Mut1, which abolishes transcription by changes in the untranslated mRNA leader. The Mut8 mutation abolishes *carQRS* promoter activity, but surprisingly, when the bases changed in the Mut8 mutation were deleted in the Δint mutant promoter, *carQRS* promoter activity was not significantly affected. Presumably, the Δint promoter has sequence characteristics able to compensate for loss of the sequences altered in the Mut8 promoter.

While the *gufA* promoter is light induced 1.25-fold in its native locus, upon transplantation to the *attB*^{Mx8} site light inducibility is lost. This is presumably a consequence of the elevated expression of the *carQRS* promoter in the dark at the *attB*^{Mx8} site.

Attenuation of light inducibility at the *attB*^{Mx8} site. Anchoring DNA stretches such that they are unable to rotate freely can create topologically constrained domains. The plausible mechanisms for constraining DNA in a domain include the binding of DNA binding proteins (7), the presence of bends in the DNA (25), and transient attachments to the membrane due to coupled transcription and translation of flanking transmembrane protein genes (5). A plausible topological domain might be expected to occur between *gufA* and *carQRS* since both GufA and CarR are predicted to contain transmembrane helices (23) and CarR has been shown to be an integral cytoplasmic membrane protein (2). In our assays the promoters assayed were flanked by genes encoding the cytoplasmic proteins β -galactosidase and aminoglycoside phosphotransferase (conferring kanamycin resistance from Tn903). The attenuation of the light induction of the *carQRS* promoter at the *attB*^{Mx8} site (6-fold induction rather than 100-fold induction)

could have been due to the loss of this mechanism of topological constraint. While the altered activity of the *carQRS* promoter at the *attB*^{Mx8} site is a concern, different promoter probes gave consistent results (Fig. 3), and light inducibility was retained. Ideally, promoter probes would have been integrated at or near the native *carQRS* locus; however, the high levels of gene conversion in *M. xanthus* made such experiments technically impractical (35).

CarQ-dependent promoters of *M. xanthus*. The promoter of the *carQRS* operon and how it is recognized by CarQ are still poorly understood, as is the case with the promoters of most other ECF family sigma factors. While ECF sigma factor-dependent promoters share conserved sequences around the -35 position, there is very little similarity around the -10 region. In organisms possessing more than one ECF sigma factor, such a lack of similarity in promoter sequences is probably required in order to allow discrimination by different sigma factor-containing RNA polymerase holoenzymes. The Mut3 *carQRS* promoter mutation allows transcription initiation by an RNA polymerase holoenzyme with a non-CarQ sigma factor ($\alpha_2\beta\beta'\sigma^X$). Since the Mut3 mutation is close to the -10 region of the *carQRS* promoter, this observation provides further evidence which suggests that in ECF sigma factors, the -10 region is critical in promoter discrimination.

The *crtI* promoter is the only other promoter in *M. xanthus* known to be CarQ dependent (11), but it, unlike the *carQRS* promoter, cannot be transcribed in vitro by using $\alpha_2\beta\beta'\sigma^{\text{CarQ}}$ (2). Another difference is the requirement in vivo for nutrient exhaustion before the *crtI* promoter can be induced by light (9). These in vitro and in vivo characteristics could be explained by the different structural characteristics of the promoters. In contrast to the *carQRS* promoter, 54 bp of upstream DNA is sufficient for *crtI* promoter activity (22), and there are significant differences in the -35 region (Fig. 1).

In this study we began to characterize the *carQRS* promoter region by determining the minimal promoter region, by assessing areas of importance within the promoter, and by identifying codependence of the *carQRS* and *gufA* promoters. A framework is thus provided for future experiments to address the following key specific questions. Where is the IhfA binding site(s)? Which sigma factors are responsible for the transcription of *gufA* and Mut3 and for the background levels of *carQRS* promoter activity observed at *attB*^{Mx8}? What is the mechanism by which CarQ can attenuate the activity of mutant promoters? Is there an enhancer sequence downstream of the *carQRS* transcriptional start site? Ongoing work in our laboratory aims to address these questions and further elucidate the structural requirements of CarQ-dependent promoters in *M. xanthus*.

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