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Genetics

Light induction of gene expression in *Myxococcus xanthus*

(carotenoid mutants/blue light/prokaryotic promoter/*lacZ* fusion)

JOSEFA M. BALSALOBRE, ROSA M. RUIZ-VAZQUEZ, AND FRANCISCO J. MURILLO*

Departamento de Genética, Facultad de Biología, Universidad de Murcia, Murcia, Spain

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ABSTRACT **The synthesis of carotenoids by *Myxococcus xanthus* requires illumination with blue light. Mutations at two loci (*carA* and *carR*) remove the blue-light requirement and cause constitutive production of carotenoids. Mutations at a different locus (*carB*) prevent carotenogenesis in both wild-type and constitutive mutant strains. We describe here three independent *car* mutations induced by insertion of Tn*5 lac*, a transposon that carries a transcriptional probe for exogenous promoters. All three transposon insertions block carotenogenesis even in constitutive mutant strains. One insertion is in a previously unknown *car* gene and the other two are in the *carB* locus. One of the *carB* insertions expresses β-galactosidase at very low levels in the dark but is strongly activated by light. When this Tn*5 lac* insertion is introduced in *carA* or *carR* mutants it expresses β-galactosidase in dark- as well as light-grown cells. We conclude that carotenogenesis in *M. xanthus* is activated at the level of transcription by a light-induced mechanism in which the *carA* and the *carR* loci (or their gene products) take part. The potential usefulness of *M. xanthus* as a simple and sensitive tool for studies in photobiology is discussed.**

Blue light is an activator, in both prokaryotic and eukaryotic organisms, of diverse metabolic, developmental, and behavioral effects. These effects are being used to study the molecular mechanism of signal detection, processing (photoreceptors and internal transducers), and response (see ref. 1 for an overview of blue-light effects).

One actively studied response to blue light is the photoinduction of carotenoid synthesis, particularly in fungi such as *Neurospora crassa* (2) and *Phycomyces blakesleeanus* (3). Blue light is also required for the production of carotenoids in the myxobacteria and seems to affect other functions in these cells as well (4–6). Because of their relatively complex life cycle, myxobacteria have attracted attention over the last few years. Sophisticated genetic tools have been developed to study these organisms, especially *Myxococcus xanthus* (7). Due to the relative ease of genetic and biochemical studies in this species it is a good choice for molecular studies of the photoinduction of carotenogenesis and perhaps for other light-inducible phenomena.

We have shown that photoinduction of carotenogenesis in *M. xanthus* involves relatively few genetic elements (8). Several constitutive mutants have been isolated in which carotenogenesis no longer depends on light. One constitutive mutation falls in a locus called *carA* and the others cluster in a second locus, unlinked to *carA*, called *carR*. Another type of carotenoid mutant is known in *M. xanthus*. A particular insertion of the transposon Tn*5*, at the site ΩDK2836, blocks carotenoid synthesis in otherwise wild-type strains, and in all of the *carA* or *carR* constitutive mutants. The locus into which Tn*5* has inserted is linked to *carA*, but it is distinct from *carA* and is called *carB*. *carB* may code for a positive regulatory element or for an enzyme acting early in the metabolic pathway (ref. 8 and Fig. 1).

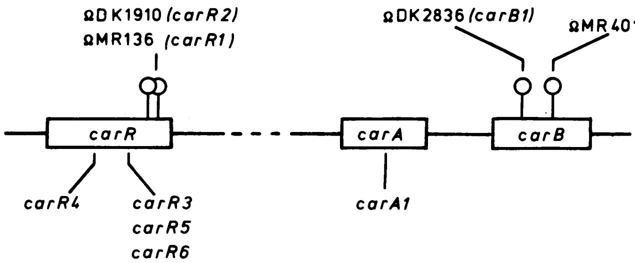

FIG. 1. Genetic map of mutations affecting carotenogenesis in *M. xanthus*. The relative order of the mutations has been drawn (not to scale) by using data of transductional genetic analysis reported by Martinez-Laborda *et al.* (8) and in this paper. Two unlinked chromosomal regions are represented. One of them contains the *carR* locus and the other the two linked loci *carA* and *carB*. Underneath, allelic numbers of mutagen-induced mutations are indicated. For transposon mutations (○), both the notation of the insertion locus and the allelic number are given. It has not been established whether ΩDK1910 and ΩMR136 are inserted at different points (8).

We have used the transposon Tn*5 lac* to investigate light-controlled gene expression in *M. xanthus*. Tn*5 lac* codes for resistance to kanamycin (Km^R) and carries a *lacZ* transcriptional probe for exogenous promoters (9). We describe three independent Tn*5 lac* insertions. One has inserted into a previously unknown *car* gene and two others are inserted into the *carB* locus. One of the latter (ΩMR401::Tn*5 lac*) has fused *lacZ* gene expression to a light-inducible promoter. β-Galactosidase expression from this fusion strain is also controlled by *carA* and *carR*. The data strongly suggest that light induces carotenoid synthesis in *M. xanthus* at the transcriptional level. Simple regulatory models that are compatible with these data are presented.

MATERIALS AND METHODS

Bacteria and Transductional Analysis. *M. xanthus* strains are described in Table 1. DK1050 is a standard strain showing normal light-inducible carotenogenesis. When grown in the dark, DK1050 forms yellow colonies due to the accumulation of a noncarotenoid pigment (10). In the light, DK1050 colonies become red, due to the accumulation of red carotenoids. Other strains carry mutations in *car* genes, as described in Table 1. Three Tn*5* insertion mutations, those present in MR140, MR144, and MR148, have also had their Tn*5* (Km^R) replaced at the same site by Tn*5*-132, which codes for resistance to oxytetracycline (Tc^R). Other *car* mutations have been mutagen induced. A constitutive mutant (*carA* or *carR*) produces carotenoids independently of the presence of

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Abbreviations: Km^R, kanamycin-resistant or resistance; Tc^R, oxytetracycline-resistant or resistance; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

*To whom reprint requests should be addressed.

Table 1. Description of *M. xanthus* strains

Strain	Genotype*	Light-inducible carotenogenesis phenotype
DK1050	Wild type	Normal
MR7	*carA1* (UV)	Constitutive
MR148†	*carB1* (ΩDK2836::Tn*5*-132)	Negative
MR401	*carB2* (ΩMR401::Tn*5 lac*)	Negative
MR402	*carB3* (ΩMR402::Tn*5 lac*)	Negative
MR403	*carC1* (ΩMR403::Tn*5 lac*)	Negative
MR140	*carR1* (ΩMR136::Tn*5*-132)	Constitutive
MR144	*carR2* (ΩDK1910::Tn*5*-132)	Constitutive
MR151	*carR3* (UV)	Constitutive
DK406	*carR4* (NTG)	Constitutive
DK718	*carR5* (UV)	Constitutive
DK2834	*carR6* (UV)	Constitutive
MR154	*carB1* (ΩDK2836::Tn*5*-132), *carR3*	Negative

*The origin of *car* mutations is indicated in parenthesis. UV and NTG stand for ultraviolet light and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, respectively. For transposon mutations, notations of the insertion loci are indicated. Tn*5*-132 codes for resistance to oxytetracycline (Tc^R) and Tn*5 lac* codes for resistance to kanamycin (Km^R). More details on the origin of the strains can be found in ref. 8 or this study.

†MR148 was used here only as donor in a cross to introduce *carB1* into MR151. MR154 was obtained in this way and was preferred for linkage studies involving *carB1* (Table 2) because it produces a higher number of transductants.

light, so that their colonies are red both in the dark and in the light. Strains carrying mutations at the *carB* or *carC* loci lack the ability to produce red carotenoids and form yellow colonies with or without light (this study; ref. 8).

Cells were grown in CTT medium (11) at 33°C by shaking (250 rpm) a 10-ml culture in 50-ml flasks kept in the dark. For plating, bottom agar and soft agar were CTT containing 1.5% and 0.7% Difco agar, respectively. When required, kanamycin or oxytetracycline was added to the media as previously described (8). Cultures were illuminated by placing them 50 cm from a battery of six 20-W fluorescent lamps (Osram L20/10S daylight).

All transductions were carried out with Mx4-LA27, a generalized transducing phage for *M. xanthus* that contains about 62 kilobases (kb) of DNA (12, 13).

Isolation of Tn*5 lac* Insertions and Enzyme Assay. Parental strain DK1050 was infected with P1::Tn*5 lac* and Km^R colonies were selected as described by Kroos and Kaiser (9). These colonies were later screened for mutant phenotypes in the photoinduction of carotenoids or for light-inducible expression of β-galactosidase (see *Results*). Two methods were used to detect β-galactosidase production. For screening purposes, colonies were transferred to CTT plates containing 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) at 40 μg/ml and, after incubation, colonies were examined for the blue color (indigo) formed by the enzymatic cleavage of X-Gal. For quantitative studies of β-galactosidase synthesis, cells were sedimented, washed, and resuspended in buffer Z (14). Extracts prepared by sonication were then assayed for their ability to cleave *o*-nitrophenyl β-D-galactoside (14), and their protein content was determined (15). β-Galactosidase specific activity is expressed as nmol of *o*-nitrophenol produced per min per mg of protein.

RESULTS

Isolation and Genetic Analysis of Tn*5 lac* Insertion Strains. Many strains with random Tn*5 lac* insertions in parental strain DK1050 were isolated. Then, two different strategies were employed to identify those Tn*5 lac* insertions at loci involved in the response to blue light. In the first strategy we screened for Tn*5 lac* insertion strains that had a mutant color phenotype associated with carotenogenesis (see *Materials and Methods* for a description of parental and mutant phenotypes). Km^R colonies from random Tn*5* insertions were transferred in duplicate to two CTT plates, both of which were incubated in the dark for 24 hr. One member of each pair of plates was then exposed to light. After 2 more days of incubation, all plates were examined for colony color. Of 2519 independent colonies tested, two failed to become red in the presence of light. These two mutants were purified by single colony isolation and named MR402 and MR403. No mutants that were red in the dark were found in this screen.

The second strategy was a direct screen for light-dependent β-galactosidase production. Individual Km^R colonies from random Tn*5 lac* insertions in DK1050 were transferred to duplicate X-Gal plates, only one of which was exposed to light after 24 hr of dark incubation. One day later, sister colonies on light and dark plates were examined for a blue color. The intense blue of indigo (the X-Gal cleavage product) masks the red or yellow of *M. xanthus* colonies. A total of 1134 Km^R colonies (different from those used in the first strategy) were examined. One colony was picked because it appeared to produce more β-galactosidase in the light than in the dark. It was purified and named MR401. MR401 was also found to be incapable of light-induced carotenoid production since its colonies remain yellow when grown on CTT plates in the light.

The color phenotypes of MR401, MR402, and MR403 resemble the phenotype of the *carB1* mutant (Table 1). We therefore wished to investigate the genetic linkage between each of the three Tn*5 lac* insertions and the *carB* locus. For this purpose, Km^R from each of MR401, MR402, and MR403 was transduced into a strain carrying insertion ΩDK2836::Tn*5*-132 (Tc^R) and the transductants were tested for loss of Tc^R. The results, presented in Table 2 (crosses 1–3), show a strong linkage between ΩDK2836::Tn*5*-132 and the Tn*5 lac* insertion loci in MR401 and MR402. In contrast, the Tn*5 lac* insertion in MR403 is not linked to *carB* and it is also not linked to *carR* (Table 2, crosses 4 and 5). Accordingly, strain MR403 must be mutated (by insertion of the transposon) in a previously unidentified *car* gene. We call this locus *carC*.

Km^R was also transduced from each of MR401 and MR402 as donors into *carA* and *carR* constitutive mutants as recipients (Table 1). In each of these transduction experiments, more than 50 Km^R transductants were examined, and all of them formed yellow colonies both in the dark and in the light. The Tn*5 lac* insertions in MR401 and MR402 give the same response to constitutive mutations as the *carB1* mutant. Thus by linkage and by response to constitutive mutations, MR401 and MR402 behave as *carB* mutants. Similar results were obtained when the Tn*5 lac* insertion of MR403 was transferred into MR7 and several of the constitutive *carR* mutants. This leads to a proposal for the possible function of gene *carC* similar to that already made for the *carB* locus (see introduction).

β-Galactosidase Expression. MR401, MR402, and MR403 were cultured in the dark, in liquid CTT medium, until they reached the late exponential phase of growth (around 10^9 cells per ml). Then the cultures were divided in two, and one was kept in the dark and the other was exposed to the light. At 1-hr

Table 2. Linkage of Tn*5 lac* insertions

Cross			Km^R transductants			Cotransduction frequency, %
Donor	×	Recipient	Total	Tc^R	Tc^S	
MR401		MR154	94	4	90	96
MR402		MR154	828	22	806	97.3
MR403		MR154	424	424	0	<0.2
MR403		MR140	224	224	0	<0.4
MR403		MR144	219	219	0	<0.4

Tc^S, oxytetracycline-sensitive.

intervals, samples were taken from each culture for quantitative assay of β-galactosidase. As can be seen in Fig. 2, the enzyme activity was very low in MR402 and MR403 and no differences were observed for these strains between dark-grown and light-grown cultures. β-Galactosidase activity was low for the dark-grown culture of MR401, but it increased very rapidly after illumination. Evidently, insertion of Tn*5 lac* in MR401 has placed the *lacZ* gene close to a light-inducible promoter and in the proper orientation for β-galactosidase expression.

The effect of *carR* mutations on the activity of the light-inducible promoter was examined next. β-Galactosidase activity was measured in a set of strains having the MR401 Tn*5 lac* insertion and various *carR* mutations. These strains were Km^R transductants from experiments described above in which a MR401 donor and the series of *carR* insertion mutants as recipients were employed. Because these transductions utilize the homology of *Myxococcus* DNA on both sides of the Tn*5 lac* insertion in MR401 for recombination with the recipient, the site and orientation of Tn*5 lac* are the same in the transductants as in MR401. As shown in Table 3, all the *carR* transductants expressed a higher level of β-galactosidase in the dark than MR401 itself. Moreover, the high level of β-galactosidase was not affected by light, indicating that the formerly light-inducible activity of the promoter has become constitutive in the *carR* mutant background. The time course of the expression of β-galactosidaseis shown in Fig. 3 for strains MR401 and MR406. The latter strain carries the MR401 Tn*5 lac* insertion and the mutation *carR3*. In MR401 (Fig. 3*A*), the β-galactosidase specific activity remained low unless the culture was exposed to the light. However, in MR406 (Fig. 3*B*), the β-galactosidase increased steadily with cell growth, and the rate of increase was not altered by exposure to light.

The effect of the *carA* constitutive mutation on the light-inducible promoter was also examined. For this purpose, a Km^R transductant from the cross Mx4(MR401) × MR7 might be used, but since the MR401 Tn*5 lac* insertion locus is linked to *carA* (Fig. 1 and Table 2), two different genotypes are expected to arise from the cross. Some of the transductants should retain the *carA1* allele, while others should gain the $carA^+$ allele from the MR401 donor along with the selected Km^R. In agreement with these expectations, two classes of Km^R colonies were obtained when MR401 Tn*5 lac* insertion was transduced into MR7. In particular, 69 colonies representing independent transductants were transferred to duplicate X-Gal plates; one plate was incubated in the dark and the other in the light. Twenty-nine colonies behaved like the MR401 parent, showing a much higher intensity of blue color in the light than in the dark. The remaining 40 colonies showed the same high intensity of blue color in the light and in the dark. The members of the first class (29/69) have apparently gained the $carA^+$ allele, and those of the second

Table 3. β-Galactosidase expression in various *M. xanthus* strains

		β-Galactosidase, nmol·min^{-1}·mg^{-1}	
Strain	Genotype	Dark	Light
MR401	ΩMR401::Tn*5 lac*	9	33
MR404	*carR1*, ΩMR401::Tn*5 lac*	279	262
MR405	*carR2*, ΩMR401::Tn*5 lac*	237	243
MR406	*carR3*, ΩMR401::Tn*5 lac*	77	78
MR407	*carR4*, ΩMR401::Tn*5 lac*	87	73
MR408	*carR5*, ΩMR401::Tn*5 lac*	37	37
MR409	*carR6*, ΩMR401::Tn*5 lac*	17	22
MR410	*carA1*, ΩMR401::Tn*5 lac*	46	43
MR411	*carB1*, *carR3*, ΩMR401::Tn*5 lac*	6	7

Each strain was grown in the dark until late exponential phase, then the culture was divided in two, and one was kept in the dark while the other was exposed to light. Enzyme activity was assayed after additional incubation for 3 hr.

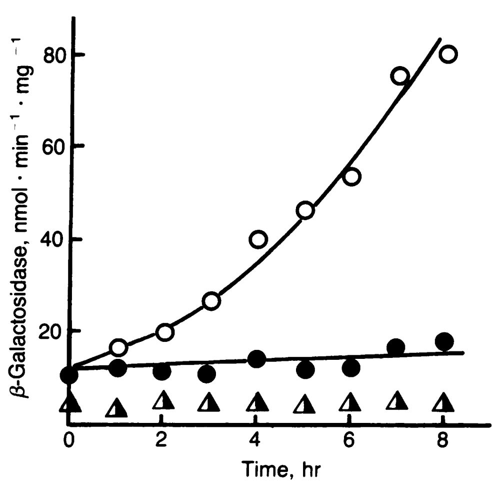

FIG. 2. β-Galactosidase activities of strains MR401, MR402, and MR403. Time zero indicates the moment at which the dark-grown cultures were divided in two, one to be kept in the dark and the other to be exposed to the light. For MR401, ●, dark and ○, light. The same symbol (▲) is used for dark and light cultures of both MR402 and MR403 strains, whose β-galactosidase specific activities varied between 2.5 and 4.5 nmol·min^{-1}·mg^{-1}.

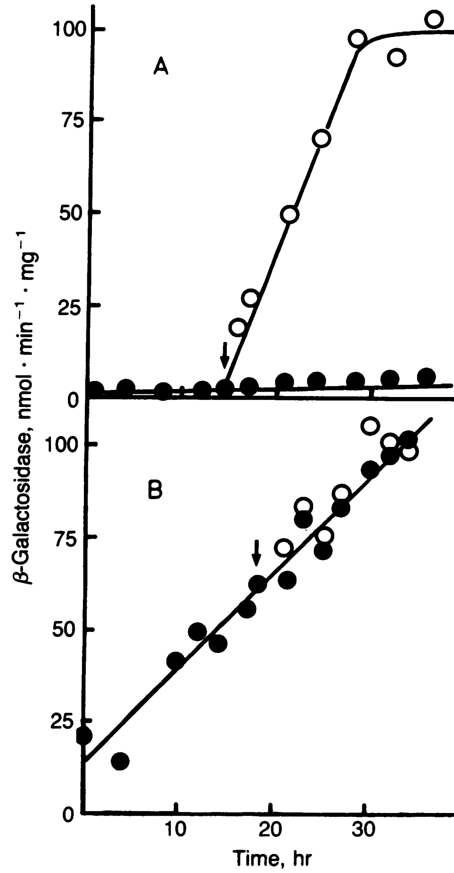

FIG. 3. Time course of β-galactosidase activity in strains MR401 and MR406. Zero time indicates the moment at which dark, early stationary cultures of MR401 (*A*) or MR406 (*B*) were diluted 20-fold in fresh medium and incubated in the dark. At the time indicated by the arrows (corresponding to a density of 10^9 cells per ml) the cultures were divided in two, one for dark (●) and the other for light (○) incubation. Stationary phase was reached at the 22 hr (MR401) and 27 hr (MR406).

class (40/69) have apparently retained the *carA1* allele, which is responsible for constitutive expression of the *lacZ* gene. This result was confirmed by the quantitative assay of β-galactosidase specific activity of strain MR410 (Table 3).

We have previously shown that the cotransduction frequency of *carA1* and *carB1* is about 70% (8). The experiment just described shows that the frequency of cotransduction between ΩMR401::Tn*5 lac* and *carA1* is around 42%. These data imply that the Tn*5 lac* insertion in MR401 is farther from *carA1* than from *carB1*, suggesting the order *carA1–carB1–*ΩMR401::Tn*5 lac*. We found that the *carB1* mutation (an insertion of Tn*5*-132 in *carB*) decreases the β-galactosidase level of MR401::Tn*5 lac* to 1/10th (Table 3, strain MR411). Strain MR411 also carries the constitutive mutation *carR3*. If transcription starts on the *carA* side of *carB*, it would be interrupted by the *carB1* insertion before it reaches the reporter *lacZ* gene in MR401::Tn*5 lac*.

DISCUSSION

Control of carotenoid production in *M. xanthus* can be summarized as follows: Carotenoid synthesis is induced by blue light (4, 5). Mutations at two unlinked loci, *carR* and *carA*, render carotenoid production light independent, or constitutive (8). The number of genes at the *carR* and *carA* loci is unknown, but several independent *carR* mutations have been found, among them point mutations and Tn*5* insertions (8). Another Tn*5* insertion, *carB1*, which is linked to *carA*, prevents the accumulation of carotenoids, in wild-type as well as constitutive backgrounds. The arrangement of these loci, derived from transductional crosses, is shown in Fig. 1.

We report here the isolation of three strains of *M. xanthus* carrying independent Tn*5 lac* insertions at loci involved in the light-induced accumulation of carotenoids. Two of the insertions, ΩMR401 and ΩMR402, have the (carotenoid-negative) CarB phenotype and are in fact very tightly linked to *carB1*. The third Tn*5 lac* insertion, ΩMR403, which also is carotenoid-negative, identifies a locus, *carC*, unlinked to the others. As proposed for *carB*, the gene *carC* might encode either a positive regulatory element or an enzyme acting in the biosynthetic pathway before the first red carotenoid is formed.

With transposon Tn*5 lac* inserted at ΩMR401, β-galactosidase activity is very low in the dark but is strongly stimulated by light. Tn*5 lac* was designed to make transcriptional, but not translational, fusions. The probe gene retains the normal translation start signal and is preceded by translation stop codons in all three reading frames (9, 16). One interpretation of these results is that expression of the *carB* locus depends on a promoter that is activated by light. (Tn*5 lac* insertion ΩMR402, also at *carB*, may be inserted in the opposite orientation, which is inappropriate for β-galactosidase expression.)

The *carA* or *carR* mutations result in the light-independent expression, at high level, from the normally light-inducible promoter. Therefore, the *carA* and *carR* loci, or their gene products, regulate the activity of that promoter. Since *carR* is not linked to *carB*, *carR* may encode a trans-acting regulator. It could, for example, encode a repressor that is, in the wild type, inactivated by light. The *carR* constitutive mutants would fail to produce a functional repressor. Recently, D. A. Hodgson (personal communication) has obtained evidence for two different functions at *carR*, one of them a repressor in the dark of the other function, an activator of the carotenogenesis. Different *carR* mutations are observed to give rise to different constitutive levels of β-galactosidase (Table 3). One possible interpretation of this observation is that different mutations alter the normal function(s) of *carR* in different ways.

The second locus of mutation to the constitutive state, *carA*, is linked to the light-inducible locus *carB*. Perhaps *carA* is a cis-acting element—for example, a promoter, operator, or both for light control of the expression of gene *carB*. The low β-galactosidase activity of the strain MR411 does suggest that transcription starts on the *carA* side of *carB*, in accord with these possibilities.

Induction by blue light of the expression of specific genes has recently been reported in higher plants (17, 18), but previously only indirect evidence has been found in microorganisms (19). Clearly, more experiments are required in *M. xanthus* to establish the regulatory mechanism suggested above for induction of gene expression by blue light. We emphasize, however, that existing data for production of carotenoids and β-galactosidase expression fit regulatory models like those recognized in *Escherichia coli* for chemically induced (or repressed) operons (20). This consideration, together with the simplicity of the cell organization and the powerful *in vivo* and *in vitro* genetic tools available for *M. xantus*, raises the hope that a molecular understanding of the regulation of gene expression by blue light can be obtained. The light response in strain MR401, detected with ease and sensitivity by the β-galactosidase assay, may provide a tool for the study of other photobiological problems. The receptor and transducers of the blue-light signal, whose molecular identities and modes of operation have remained elusive in many other systems (1), may be investigated this way.

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