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Regulation of *ubiG* Gene Expression in *Escherichia coli*

ISIDRE GIBERT, MONTSERRAT LLAGOSTERA, AND JORDI BARBÉ*

Department of Genetics and Microbiology, Autonomous University of Barcelona, Bellaterra, Barcelona, Spain

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To study the regulation of the expression in *Escherichia coli* of the *ubiG* gene, which codes for the last enzyme in the pathway of ubiquinone biosynthesis, a fusion between the *ubiG* and *lacZ* genes was constructed in vitro. The results showed that (i) the expression of the *ubiG* gene was higher under aerobic conditions than under anaerobic growth conditions, (ii) the presence of glucose in the culture medium decreased the transcription of the *ubiG* gene, and (iii) *cya* and *crp* mutants exhibited lower levels of *ubiG* gene expression than the wild-type strain. The addition of cyclic AMP increased the expression of the *ubiG* gene in both *cya* and wild-type strains but not in a *crp* mutant. This fact suggests that the cyclic AMP receptor protein-cyclic AMP complex positively modulates *ubiG* gene transcription. It was also determined that the transcription of the *ubiG* gene was in the counterclockwise direction on the *E. coli* map.

Quinones are the links between the dehydrogenases and the next protein component in the sequence of the respiratory chains. In *Escherichia coli*, the protein can be cytochrome oxidase, nitrate reductase, or fumarate reductase (11). *E. coli* is capable of synthesizing two quinones: menaquinone-8 and ubiquinone-8 (10).

Five genes code for the biosynthesis of menaquinone: *menA* (min 88), *menB* and *menC* (min 48), and *menD* and *menE*, whose map positions have not been determined yet (1, 2). On the other hand, the eight genes that determine the synthesis of ubiquinone in *E. coli* have been mapped: *ubiA* and *ubiC* (min 91); *ubiB*, *ubiD*, and *ubiE* (min 85); *ubiF* (min 15); *ubiG* (min 48); and *ubiH* (min 62) (1).

The ratio of menaquinone to ubiquinone in cells is variable, depending on the growth conditions (18). Typically, with high aeration and at the logarithmic phase, ubiquinone is at a higher concentration than menaquinone, whereas in anaerobic cultures, the concentration of ubiquinone decreases dramatically while the level of menaquinone increases (4).

Nevertheless, nothing is known to us about the control mechanisms of any of the several genes involved in either menaquinone or ubiquinone synthesis. In this work, we have studied, through the construction of a *ubiG-lacZ* fusion, the regulation of the expression of the *ubiG* gene, which codes for the final enzyme in the ubiquinone biosynthesis pathway that catalyzes ubiquinone-8 production from 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinone.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Bacterial strains and plasmids used in this work are listed in Tables 1 and 2.

Aerobic cultures were grown at 37°C with shaking in either LB rich medium (13) or AB minimal medium (9) supplemented with Casamino Acids at 0.4% (wt/vol) and glucose or glycerol at 0.2% (wt/vol). Malate, citrate, and succinate, when necessary, were used at 0.4% (wt/vol). LB plates containing 20 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml were used to screen colonies for β-galactosidase production.

Anaerobic cultures were grown in completely filled screw-cap bottles containing AB minimal medium with 50 mM KNO_3, 1 μM Na_2SO_3, and 1 μM Na_2MoO_4 and supplemented with Casamino Acids and glycerol. They were incubated at 37°C with gentle shaking by a magnetic stirrer. With anaerobic cultures, nitrate respiration was periodically determined by nitrite production by a modification of the method of Nicholas and Nason (15). Culture samples were mixed vigorously with 4 ml of reagent containing 0.25% sulfanilic acid, 0.15% α-naphthylamine, and 15% acetic acid, and A_{540} was measured 30 min later. Samples for analysis of nitrite or other parameters (such as optical density and β-galactosidase levels) were withdrawn with a syringe after nitrogen was injected into the culture with a hypodermic needle and were processed immediately.

Antibiotics were used at the following concentrations: streptomycin, 75 μg/ml; ampicillin, 50 μg/ml; and tetracycline, 15 μg/ml.

β-Lactamase assay. A volume of 10 ml taken from the desired culture was chilled on ice and centrifuged. The cells were washed, suspended in 0.5 ml of phosphate buffer, and sonicated on ice by five cycles of sonication (10 s with a Sonifier [Branson Sonic Power Co.] at setting 3, with 45 s of cooling). The broken cells were centrifuged at 13,000 × *g* for 30 min at 4°C, and the resulting supernatant fluid was used as the crude enzyme. Usually, 5 to 40 μl of extract per ml of substrate solution was required.

β-Lactamase activity was determined by a spectrophotometric method (19) that measured the decrease in A_{235} (23) of a solution of ampicillin (100 μM). Specific activity was calculated as micromoles of substrate hydrolyzed per minute at 22°C per milligram of total protein (5), with bovine serum albumin as the standard.

β-Galactosidase assay. The β-galactosidase assay was performed as described by Miller (14). Enzyme concentrations (units per milliliter) were calculated from the formula given by Casaregola et al. (8).

DNA techniques. Plasmid DNA was isolated by the alkaline extraction procedure of Birnboim and Doly (3) and purified by banding in a CsCl-ethidium bromide gradient as previously described (13). The ethidium bromide was removed with butanol, followed by exhaustive dialysis. Restriction enzymes, T4 DNA ligase, and alkaline phosphatase were purchased from Boehringer Mannheim Biochemicals and were used as recommended by the suppliers. DNA fragments after enzyme digestion were purified in 0.8%

* Corresponding author.

TABLE 1. Strains of *E. coli* used in this work

Strain	Genotype	Source
MC1061	*araD139* Δ(*ara leu*)*7697* Δ*lacX74* *galU galK*	M. Casadaban
TP2100	*xyl ilvA argH1* Δ*lacX74*	J. R. Guest
TP2000	TP2100 Δ*cya*	J. R. Guest
TP2139	TP2100 Δ*crp*	J. R. Guest
AN86	Hfr *metB ubiG46*	F. Gibson
UA4399	AN86(pPS2)	This work
UA4479	MC1061(pUA54)	This work
UA4480	TP2000(pUA54)	This work
UA4481	TP2100(pUA54)	This work
UA4482	TP2139(pUA54)	This work
UA4483	AN86(pUA54)	This work

(wt/vol) agarose gels by electroelution. Plasmid DNA transformation was carried out as previously described (13).

RESULTS AND DISCUSSION

***ubiG-lacZ* fusion construction.** pPS2 is a pBR322 derivative plasmid harboring a *Pst*I chromosomal fragment of 12 kilobases (kb) inserted in the unique *Pst*I site (Fig. 1) (16). This chromosomal fragment of pPS2 contains *nrdAB* and *ubiG* genes. This plasmid can support the growth at 42°C of temperature-sensitive *nrdAB* mutants (16). It also enables the UbiG$^-$ mutant AN86 (21) to grow in succinate (data not shown). Moreover, *nrdAB* and *ubiG* genes do not overlap, because the frequency of their cotransduction by P1 is about 10% (12). For all these reasons, we used plasmid pPS2 to obtain an in vitro fusion between the *ubiG* and the *lacZ* genes.

Plasmid pPS2 was digested by *Bam*HI, and the fragment of 5.4 kb was ligated in the *Bam*HI sites of plasmids pSKS107, pFR97, and pFR109. These plasmids are vectors for hybrid protein–β-galactosidase gene fusions, and they contain a polylinker with a *Bam*HI site upstream of a promoterless *lac* gene (20). Further, each of plasmids pSKS107, pFR97, and pFR109 presents a different reading frame of *lacZ* gene after *Bam*HI digestion (20).

Cells transformed with pSKS107 derivative plasmids did not give any blue colony on X-Gal plates, regardless of the orientation of the cloned fragment. On the other hand, cells transformed with pFR97 or pFR109 derivative plasmids produced both blue and white colonies. Cells harboring the pFR109 derivative showed a blue color only when the orientation of the 5.4-kb fragment produced a fusion between the *nrdA* and the *lacZ* genes. Likewise, cells containing the pFR97 derivatives gave a blue color only when the orientation of the fragment subcloned was that shown in Fig. 2. Then, the plasmid designated pUA54 (Fig. 2) was introduced by transformation in the UbiG$^-$ AN86 strain, and the ability of these transformed cells to grow with succinate as the carbon source was tested. Whereas the AN86(pPS2) strain used succinate, AN86(pUA54) cells did not grow under these conditions.

Digestion of pPS2 plasmid by the *Bam*HI enzyme also generated a fragment of 4.1 kb containing both chromosomal and pBR322 DNA (Fig. 1). To corroborate that pUA54 plasmid presented a *ubiG-lacZ* fusion, two factors still had to be confirmed: (i) that this 4.1-kb fragment did not contain a functional *ubiG* gene, and (ii) that this fragment did not have a promoter at its chromosomal end. In this respect, the 4.1-kb *Bam*HI fragment of plasmid pPS2 (Fig. 1) was unable

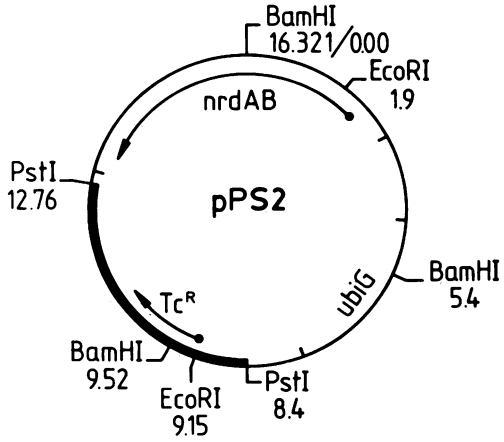

FIG. 1. Structure of pPS2. ——, pBR322 DNA; ——, chromosomal DNA. The directions of known transcriptions are indicated (arrows). Only the positions of the restriction sites relevant as landmarks to plasmid construction are given. For the restriction sites indicated, all sites of that type on the plasmid are shown.

TABLE 2. Plasmids used in this work

Plasmid	Genetic characteristics	Reference
pPS2	Tc^r UbiG$^+$ NrdAB$^+$	16
pSKS107[a]	Amp^r Lac$^-$ (1)[b]	20
pFR97[a]	Amp^r Lac$^-$ (3)[b]	20
pFR109[a]	Amp^r Lac$^-$ (2)[b]	20
pSKS104[c]	Amp^r Lac$^+$ (3)[b]	20
pSKS105[c]	Amp^r Lac$^+$ (1)[b]	20
pSKS106[c]	Amp^r Lac$^+$ (2)[b]	20
pUA54	Amp^r *ubiG* mutation	This work

[a] Vectors without the *lac* promoter for forming hybrid protein–β-galactosidase gene fusions and containing a polylinker sequence with a *Bam*HI site upstream of the *lacZ* gene. The size of these plasmids is 9.9 kb, and their replicon is pMB1.

[b] The numbers in parentheses indicate which nucleotide of the codon follows the center of the palindromic restriction enzyme recognition sequence after *Bam*HI digestion.

[c] Vectors containing the wild-type *lac* promoter and a polylinker sequence with a *Bam*HI site between the *lacZ* translation initiation codon and the C-terminal sequence of the *lacZ* gene. The size of these plasmids is 10.1 kb, and their replicon is pMB1.

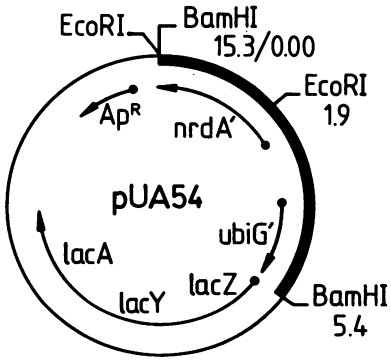

FIG. 2. Structure of plasmid pUA54 containing an in vitro fusion of *ubiG* and *lacZ* genes. The sequence components of parental plasmids were pPS2 (▭) and pFR97 (——). The directions of transcription of several genes are indicated (arrows). Only the positions of the restriction sites relevant as landmarks to plasmid construction are given. For the restriction sites indicated, all sites of that type on the plasmid are shown.

TABLE 3. Level of *ubiG* gene transcription in strain UA4479 harboring plasmid pUA54 and grown in several carbon sources

Medium[a]	β-Galactosidase (U)[b]	β-Lactamase (relative U)[c]	β-Galactosidase (U)/β-lactamase (relative U)
LB	6.2 (0.4)	1	6.2
LB + glucose	3.3 (0.3)	1.1	3
AB + glucose	3.5 (0.3)	1.1	3.2
AB + glycerol	9.3 (0.7)	1	9.3
AB + malate	8.7 (0.7)	1.2	7.3
AB + citrate	12.8 (0.9)	1.1	11.6
AB + succinate	14.1 (1.1)	1.1	12.8
AB + glycerol + nitrate	2.4 (0.2)	2	1.2
AB + glycerol + nitrate + glucose	3.2 (0.3)	1.7	1.9

[a] All cultures were grown aerobically, except those which had nitrate in the medium.

[b] The β-galactosidase assay was performed in exponentially growing cultures at concentrations of about 3×10^8 CFU/ml. All values are the averages (± standard deviation) of three independent determinations.

[c] Relative to the units of β-lactamase of the culture grown in LB medium.

to support growth in the presence of succinate if cloned in the pFR97 plasmid and introduced in the UbiG⁻ AN86 mutant. Furthermore, plasmids pSKS107, pFR97, and pFR109, harboring the 4.1-kb *Bam*HI fragment with the chromosomal end fused to the *lacZ* gene, were unable to produce any blue colonies on X-Gal.

The results cited above suggested that the *ubiG* gene of plasmid pPS2 had been cut by *Bam*HI digestion and that the *lacZ* gene was under the control of the *ubiG* promoter in plasmid pUA54. However, another explanation of our results could be that the *ubiG* gene was cotranscribed with another gene located upstream of *ubiG* and downstream from the promoter. Thus, plasmid pUA54 could have the regulatory region of *ubiG* even though this gene was not present in this plasmid. To examine this possibility, plasmids

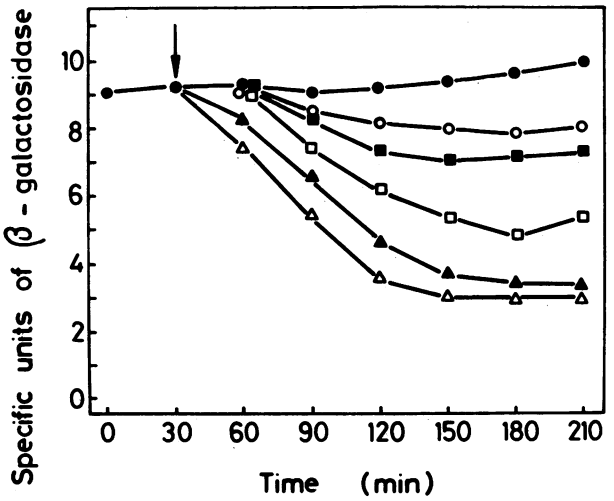

FIG. 3. Effect of glucose on the level of *ubiG* gene transcription, as indicated by β-galactosidase synthesis. Cells of the UA4479 strain harboring plasmid pUA54 were grown in AB minimal medium supplemented with glycerol at 0.4% (wt/vol) until the mid-logarithmic phase of growth (arrow), after which the culture was divided into several aliquots, and glucose was added to each aliquot at the following concentrations (percentages): 0.2 (△), 0.1 (▲), 0.05 (□), 0.01 (■), 0.005 (○), and 0 (●).

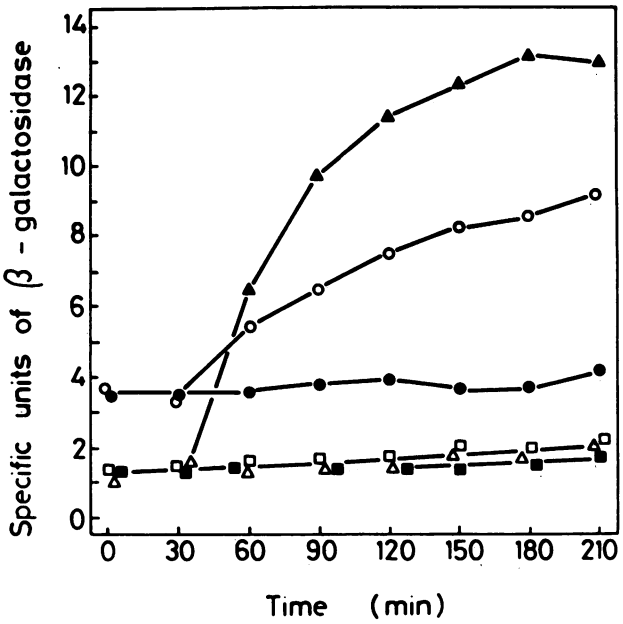

FIG. 4. Effect of 10 mM cyclic AMP on the level of *ubiG* gene transcription, as indicated by β-galactosidase synthesis in *E. coli* Cya⁻ UA4480 (▲), Crp⁻ UA4482 (■), and wild-type UA4481 (○) grown in AB minimal medium supplemented with Casamino Acids and glucose. Levels of Cya⁻ (△), Crp⁻ (□), and wild-type (●) strains without cyclic AMP added are shown as controls.

pSKS104, pSKS105, and pSKS106, each harboring a polylinker with a *Bam*HI site downstream from the *lac* promoter, were used. Each of these plasmids also generates a reading frame different from that of the *lac* promoter after *Bam*HI digestion (20). Then, the 4.1-kb *Bam*HI fragment of pPS2 was purified and cloned into the *Bam*HI site of each of plasmids pSKS104, pSKS105, and pSKS106, and any plasmid obtained in this way supported the growth of the UbiG⁻ mutant in succinate, regardless of the presence of isopropyl-β-D-thiogalactopyranoside. These results showed that the entire *ubiG* gene was not present in the 4.1-kb *Bam*HI fragment of pPS2 and that plasmid pUA54 must have contained a *ubiG-lacZ* fusion.

Furthermore, the data obtained showed that the transcription of the *ubiG* gene was in the counterclockwise direction of the *E. coli* map, because the *ubiG* and *nrdAB* genes presented opposite directions of transcription (Fig. 1 and 2) and the transcription of the *nrdAB* genes is in the clockwise direction of the *E. coli* map (1, 5).

Regulation of *ubiG* gene expression. It has been reported that the level of ubiquinone in *E. coli* can change under different growth conditions (2). For this reason, the level of expression of the *ubiG-lacZ* fusion was measured in several media. The highest expression of the *ubiG* gene was in cells grown with either citrate or succinate as the carbon source (Table 3). Cells grown in glucose presented the lowest level of *ubiG* gene expression. Likewise, cells grown anaerobically in the presence of nitrate showed a β-galactosidase level about four times lower than that for cells grown aerobically in the presence of glycerol. These values agree with previous results showing that the level of ubiquinone is dramatically lower under anaerobic growth conditions (17). Furthermore, the addition of glucose to *E. coli* cells grown in LB medium decreased *ubiG* transcription (Table 3). This fact led us to study the possible role of repression by catabolite

effect on *ubiG* gene expression. The addition of increasing concentrations of glucose induced a continuous decrease of *ubiG* gene transcription (Fig. 3). In agreement with this inhibitory effect of glucose on *ubiG* gene transcription, cyclic AMP stimulated β-galactosidase production in both *cya* and wild-type strains but not in a *crp* mutant (Fig. 4). Likewise, *cya* and *crp* mutants presented lower *ubiG* gene expressions than the wild-type strain. All these data enabled us to conclude that the transcription of the *ubiG* gene is positively modulated by the cyclic AMP receptor protein-cyclic AMP complex.

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