SHORT PAPER

An *E. coli* promoter that is sensitive to visible light

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ABSTRACT

It has been discovered that expression of promoter activity can be inhibited by visible light when specific fragments of *E. coli* DNA are inserted in a vector system designed to assay for promoter activity. These fragments have been located on regions of the *E. coli* chromosome to which no gene has been assigned to date. The effective wavelength of light that produces this phenomenon has been determined.

When studying the expression in vivo of promoters of chloroplast genes in *E. coli*, we encountered an interesting phenomenon. That is, when a particular vector carries a specific fragment of the *E. coli* genome, the reporter gene is expressed in the dark but not in the light. Fig. 1 shows the vector that we constructed and used to assay for promoter activity. A reporter gene, namely, a tRNA amber suppressor gene, was inserted in the multiple-cloning site of pUC18 [Fig. 1(a); (Nakahigashi et al., 1990)] and the *lac* promoter was deleted [Fig. 1(b)]. If a fragment of DNA, inserted in front of this reporter gene, contains a promoter, amber suppressor activity can be detected.

In this experiment, *E. coli* DNA was digested with the restriction enzyme *Bam*HI and fragments were ligated into the vector shown schematically in Fig. 1. These constructs were used to transform *E. coli* K12 strain CA274 (Russell et al., 1970) that carried lac_{am} trp_{am} mutations. Lac^+Trp^+ transformants were selected on minimal (M9) lactose plates in the dark at 25°C, and then colonies were replica-plated onto two plates, one of which was incubated in the dark and the other in the light. "Light", as used here, means constant illumination at a site 15 cm below a fluorescent light (about 7500 lux). We found three out of 500 colonies tested that did not grow in the light under these conditions. Two of them (designated ls-1 and ls-17) were further examined. Plasmids were re-isolated and
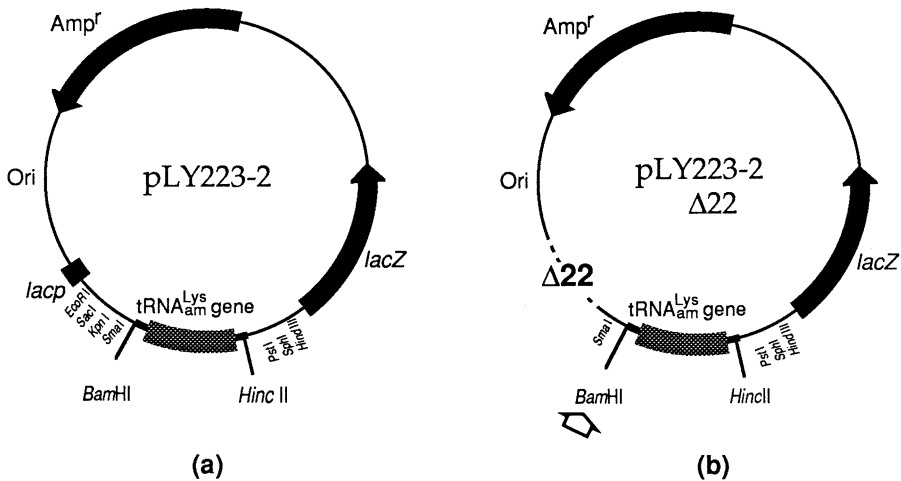
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Fig. 1. The vector used to assay for promoter activity. The gene for tRNA^{Lys} (*trnK*) in the liverwort *Marchantia polymorpha*, which carries a group II intron between the anticodon loop and the anticodon stem, as well as a mismatch (C_{27}–C_{43}) at the first pair of the anticodon stem (Ohyama et al., 1988), was artificially converted to an amber suppressor tRNA^{Lys} gene. This mutant tRNA gene was ligated downstream from the *lac* promoter on pUC18. The construction and characterization of the plasmid that carries an active amber suppressor gene [pLY223-2; (a) in Fig. 1] have been described by Nakahigashi et al. (1990). The procedures described by Yanisch-Perron et al. (1985) were used to delete the *lac* promoter on pLY223-2 [(b) in Fig. 1]. The △22 deletion extended 363 base pairs from *Kpn*I site in the mutiple-cloning site of pUC18 to the upstream region of the *lac* promoter, as confirmed by DNA sequencing. The *Bam*HI site (indicated by an open arrow) was used to insert *Bam*HI fragments from *E. coli* DNA, and the plasmids confering amber suppressor activity were selected after transformation of strain CA274 *lac*$_{am}$ *trp*$_{am}$ on minimal (M9) lactose-ampicillin plates. Even without any inserted fragment, pLY223-2 △22 showed amber suppressor activity to some extent at 37°C, but not at 25°C. This residual promoter acitivty at 37°C may be due to a readthrough of transcription from the Ori region.

Table 1. Suppression of the synthesis of β-galactosiase

Plasmid	β-galactosidase unit	
	Light	Dark
pLY223-2	30.1	31.8
pLY223-2 △22	0.18	0.16
pLY ls-1	0.25	1.32
pLY ls-17	0.21	0.72

The activity of β-galactosidase in cells of CA274 *lac*$_{am}$*trp*$_{am}$ that carried the various plasmids indicated, grown at 25°C in the light and in the dark, was measured after induction for 180 min with 5 mM isopropyl-β-D-thiogalactoside (IPTG). Toluene-treated bacteria were assayed for the rate of hydrolysis of o-nitrophenyl-β-D-galactoside and units of enzyme were calculated as described by Miller (1972).

used to transform the original strain, as well as another strain, 9829 (Nagata and Horiuchi, 1973), that carried the amber mutations. Amber suppression was again definitely inhibited by visible light. As shown in Table 1, suppression was measured by assays of β-galactosidase activity under light and dark conditions. Although not much β-galactosidase was produced, a clear difference was observed. We determined the size of the fragments that, when inserted, gave rise to the light-sensitive phenotype described above; the sizes of fragments from clones of ls-1 and ls-17 were 9 kb and 3 kb, respectively. These fragments were then inserted into another promoter-assaying vector (pKK232-8) with a gene for chloramphenicol transacetylase (CAT) as the reportor gene, which was obtained from a commercial sourse. Using this system, we were again able to detect the light-sensitive phenomenon, as shown in Fig. 2. The fact that expression of resistance to chloramphenicol was also light-sensitive confirmed what we had seen in the assays for suppressor activity.
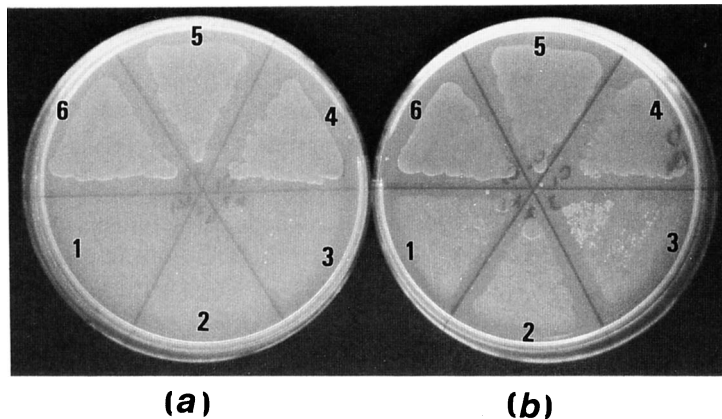

(a) **(b)**

Fig. 2. Evidence that expression of the CAT gene on plasmid pKK232-8 (purchased from Pharmacia LKB Biotechnology, Uppsala, Sweden) becomes light-sensitive as a result of insertion of the ls-1 fragment as a promoter. The ls-1 fragment or, as a control, a weak promoter (pSC9) which was constructed artificially by Aoyama and Takanami (1988) and contains the perfect consensus sequences at the −35 and −10 regions with an unsuitable spacer length (15 base pairs), was inserted into the *Sma*I site of pKK232-8 and used to transform CA274. The Amp^r Cm^r transformants were isolated in the dark and three of clones from each construct were streaked on L plates that contained 50 μg/ml of ampicillin and 15 μg/ml of chloramphenicol. The plates were incubated at 37°C overnight in the light (a) and in the dark (b). Transformants ♯1 to ♯3 are from the plasmid with the ls-1 fragment and ♯4 to ♯6 are from the plasmid with the artificial promoter.

Using Kohara's λ clones, which contain the entire chromosome of *E. coli* K12 (Kohara et al., 1987), we carried out a hybridization experiment to identify the source of the active fragments. The result is shown in Fig. 3. Fragment ls-1 is found on clones ♯549 and ♯550 (near *rrnA*, at 86 min), and fragment ls-17 is
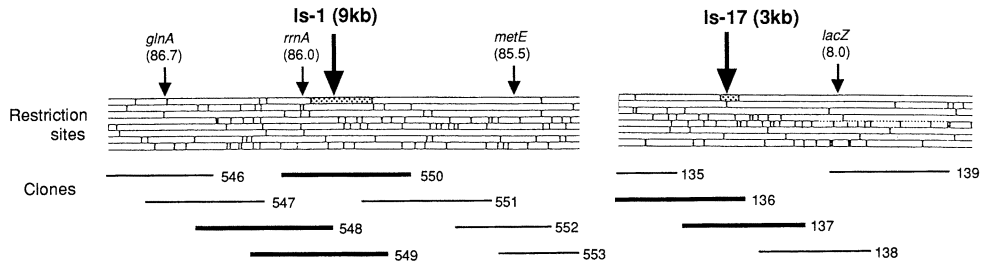
Fig. 3. Physical map locations of the ls-1 and ls-17 fragments. They were determined by plaque hybridization using "mini-set" clones (476 lambda clones covering the entire chromosome of *E. coli*) from Kohara's library (Kohara et al., 1987). Horizontal lines indicate indivisual clones of the "mini-set" and clones that hybridized with the ls-1 or ls-17 fragments are shown in bold lines. Clone ♯548 hybridized only partially. Restriction sites are shown for *Bam*HI, *Hind*III, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I and *Pvu*II (from the top line to the bottom). *Bam*HI fragments corresponding to the ls-1 and ls-17 fragments are shadowed. Well-known genes located in the flanking regions are also indicated with their genetic map positions in parentheses.

found on clones ♯136 and 137 (near *lac*, at around 7.8 min). To date, no gene has been mapped to these regions (Bachmann, 1990).

Next we examined the wavelength of visible light at which the inhibition is most effective. Cells of the bacterial strain CA274 lac_{am} trp_{am}, carrying the plasmid pLY ls-1 with the light-sentitive promoter (ls-1) and the amber suppressor gene, were grown in a minimal (M9) lactose medium at 25°C for 6 hrs under light of various wavelengths (the intensity was held constant), by use of the large spectrograph at the National Institute for Basic Biology at Okazaki. As shown in Fig. 4, it was clear that light with a wavelength of 480 nm was most effective in inhibiting growth, although light in the near-ultraviolet region was also inhibitory to some extent.

We have been sequencing both the ls-1 and ls-17 fragments, and we have narrowed the light-sentitive promoter region of the ls-1 fragment to about 0.5 kb by subcloning. Although the promoter activity of the fragments described here is very weak, only these specific fragments were associated with this light-sensitive phenomenon. At present, we cannot explain how *E. coli* absorbs visible light, how the light energy is transmitted and transformed, or how light energy affects transcription. However, since *E. coli* is the best-characterized organism in terms of both genetics and biochemistry, the discovery of this phenomenon in *E. coli* offers a simple experimental system for studies in the field of photobiology.
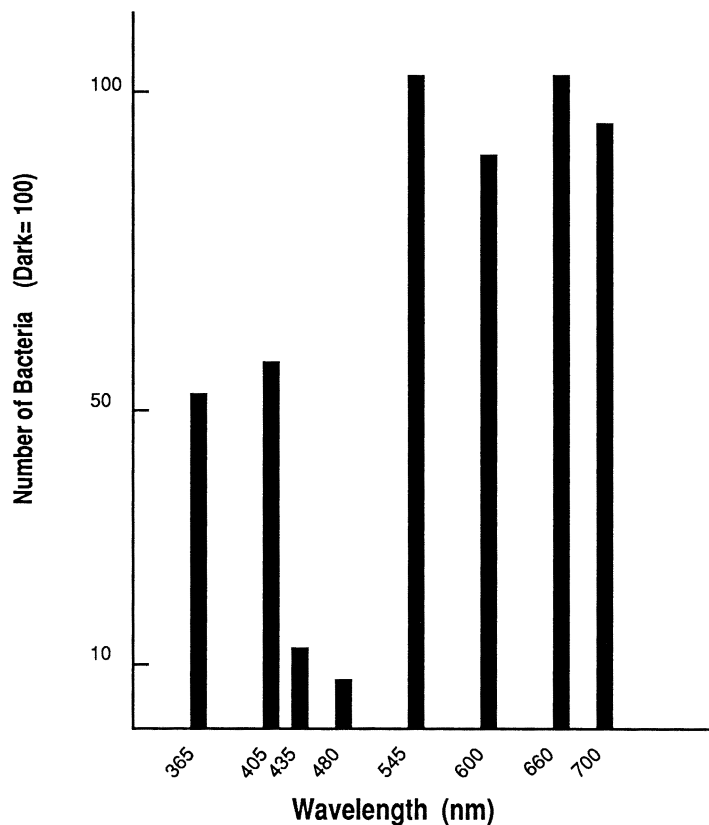
Fig. 4. Inhibition of the promoter activity by light of various wavelengths. Approximately 500 bacteria of strain CA274 lac_{am} trp_{am}, carrying the plasmid pLY ls-1 grown to logarithmic phase in 2 ml of a minimal (M9) lactose medium, were placed into a small, transparent, plastic petri dish (Nunc 35×10 mm type), and the samples were irradiated with monochromatic light from a 30 kW xenon short-arc lamp through a convex lens and appropriate neutral-density filters to adjust the fluence rates (1.5×10^{15} quanta cm^{-2}s^{-1}). The Okazaki Large Spectrograph used in this study has been described in detail elsewhere (Watanabe et al., 1982). All the procedures were carried out in a dark room at 25°C under a red safe light. Control batches of bacteria were kept in the dark at the same temperature for the duration of irradiation. After irradiation for 6 hours, the bacteria were plated on LB plates. The plates were incubated at 37°C overnight in the dark for counting of colonies. The relative extent of inhhibition of colony formation is expressed as a percentage of the result for control cells incubated in the dark.

REFERENCES

AOYAMA, T. and TAKANAMI, M. (1988) Supercoiling response of *E. coli* promoters with different spacer lengths. *Biochem. Biophy. Acta* **949**, 311–317.

BACHMANN, B. J. (1990) Linkage map of *Escherichia coli* K-12, Edition 8. *Microbiol. Rev.* **54**, 130–197.

Kohara, Y., Akiyama, K. and Isono, K. (1987) The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. *Cell* **50**, 495–508.

Miller, J. H. (1972) Assay of β-galactosidase. In: *Experiments in Molecular Genetics*, pp. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Nagata, T. and Horiuchi, T. (1973) Isolation and characterization of a temperature-sensitive amber suppressor mutant of *Escherichia coli* K12. *Mol. Gen. Genet.* **123**, 77–88.

Nakahigashi, K., Inokuchi, H. and Ozeki, H. (1990) Functional expression of the mutants of the chloroplast tRNA^{Lys} gene from the liverwort, *Marchantia polymorpha*, in *Escherichia coli*. *FEBS Letters* **265**, 59–62.

Ohyama, K., Fukuzawa, H., Kohchi, T., Sano, S., Shirai, H., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H. and Ozeki, H. (1988) Structure and organization of *Marchantia polymorpha* chloroplast genome. I. Cloning and gene identification. *J. Mol. Biol.* **203**, 281–298.

Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., Brenner, S. and Smith, J. D. (1970) Duplicate genes for tyrosine transfer RNA in *Escherichia coli*. *J. Mol. Biol.* **47**, 1–13.

Watanabe, M., Furuya, M., Miyoshi, Y., Inoue, Y., Iwahashi, I. and Matsumoto, K. (1982) Design and performance of the Okazaki large spectrograph for photobiological research. *Photochem. Photobiol.* **36**, 491–498.

Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Improved M13 phage cloning vectors and host strains in nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–119.