Photosensitivity of a protoporphyrin-accumulating, light-sensitive mutant (*visA*) of *Escherichia coli* K-12

(heme/active oxygen/protoporphyrin IX/ferrochelatase)

KENJI NAKAHIGASHI, KOICHI NISHIMURA, KAZUMASA MIYAMOTO, AND HACHIRO INOKUCHI*

Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Communicated by Julius Adler, August 19, 1991

ABSTRACT Mutations in the *visA* gene of *Escherichia coli* cause the mutant bacteria to die upon illumination with visible light. We confirmed genetically that the *visA* gene is a structural gene for ferrochelatase (protoheme ferro-lyase, EC 4.99.1.1). Since other mutations in the genes involved in the biosynthesis of heme can cure the photosensitivity, the light-induced cell death appears to be brought about by the accumulation of protoporphyrin IX, one of the substrates of ferrochelatase. When cells are illuminated with visible light, protoporphyrin IX seems to produce an active species of oxygen (probably $^1\text{O}_2$) that is harmful to the cells. This defect is the same as that associated with the human disease protoporphyria.

Erythropoietic protoporphyria, a hereditary disease of humans, causes cutaneous photosensitivity. The photosensitivity is caused by the accumulation of large amounts of protoporphyrin IX (proto IX) in erythrocytes. The accumulation of proto IX is induced by a decrease in the activity of ferrochelatase (protoheme ferro-lyase, EC 4.99.1.1), the final enzyme in the biosynthesis of heme, which incorporates ferrous iron into the tetrapyrrole ring of proto IX. When illuminated with visible light, proto IX is believed to produce an activated form of oxygen that causes various types of damage to the body. However, the exact nature of the reactions is not known yet. A relation between human porphyrias and porphyrin-accumulating mutants of *Escherichia coli* has already been mentioned (1).

We reported previously the isolation of mutants of *E. coli* K-12 that were sensitive to visible light (2). Most such mutants were defective in one gene, designated *visA*. The *visA* mutants were sensitive to visible light of about 460 nm but had no accentuated sensitivity to ultraviolet light. The *visA* gene is located at 11 min in the *E. coli* chromosome just downstream of the *adk* gene, and it encodes a protein of 320 amino acid residues. Since this protein showed 28% identity to the ferrochelatase from *Saccharomyces cerevisiae* (3), we suggested that the *visA* gene is the same gene as *hemH*, which was classically designated as encoding the enzyme.

In the present study, we have confirmed genetically that the *visA* gene does in fact encode the ferrochelatase and that the photosensitivity results from the accumulation of proto IX, as in the case of human protoporphyria. We also investigated the formation of active oxygen by photochemical reactions mediated by the endogenous proto IX.

MATERIALS AND METHODS

Bacterial Strains. All the bacterial strains used were derivatives of *E. coli* K-12 and are listed in Table 1. VS200 is a deletion mutant of the *visA* gene. Photoresistant revertants

were isolated from VS200 by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). A hemin-permeable mutant was also isolated with NTG. The $\Delta\text{sodA}::\text{lacZ}$ Cm^R marker was transduced from QC772 by P1 transduction, as described by Miller (6).

Media and Growth. The basic media used were LB and M9 minimal medium (7). Fumarate minimal medium contained mineral salts (8), 1% glycerol, and 50 mM sodium fumarate. NO_3 minimal medium contained mineral salts, 0.5% glycerol, 1% potassium nitrate, and 1 μM ammonium molybdate. When required, media were supplemented with 0.2% glucose, 0.6% sodium succinate, 20 μg of a particular amino acid per ml, 100 μg of 5-aminolevulinic acid (ALA) per ml or 10 μg of hemin per ml, unless otherwise noted. For illumination, plates were exposed to the light from two 40-W fluorescent lamps at a distance of 15 cm (about 7500 lux), and the liquid cultures in test tubes were illuminated by "a cold light" illuminator (HLS2150 type; Hoya-Schott, Tokyo). Dark controls were wrapped in aluminum foil and placed next to the experimental samples. Incubations were performed at 37°C under aerobic conditions, unless otherwise noted.

DNA Clones. λvisA is a phage clone carrying the 3.5-kilobase *EcoRI* fragment that contains the *visA* gene (2); it was constructed by insertion of the 3.5-kilobase fragment into the *EcoRI* site of $\lambda\text{gt}-\lambda\text{C}$ (9). $\lambda\Delta\text{visA}$ was constructed as follows. The *EcoRV*-*Nae* I region within the *visA* gene was deleted from the 3.5-kilobase *EcoRI* fragment, and the fragment with the deletion was exchanged for the λC fragment of the vector phage $\lambda\text{gt}-\lambda\text{C}$. Because this phage, $\lambda\Delta\text{visA}$, does not have a phage attachment site (*pop'*), it cannot integrate into the host chromosome by phage functions. All the other λ clones used have been described by Kohara *et al.* (10). Plasmid pUR290 was described by Rther and Mller-Hill (11). Plasmid pHA1 was constructed by inserting the PCR-amplified *hemA* gene, with flanking regions, into the vector pUC18. The *nfo*'-'*lacZ* fusion plasmid pH1201 (H. Ikehata and S. Yonei, personal communication) and the *katG*'-'*lacZ* plasmid pKT1033 (12) were kindly supplied by S. Yonei.

Deletion of the *visA* Gene Mediated by Phage λ . $\lambda\Delta\text{visA}$ was lysogenized into the chromosome of CA274 by homologous recombination between the cloned fragment and the chromosome. The phage was cured by heat-pulse curing (13). A deletion mutant was isolated by screening the cured isolates.

Mutagenesis with NTG. For isolation of photoresistant revertants, mutagenesis with NTG was carried out as described by Miller (6). For isolation of hemin-permeable mutants, 0.1 ml of an overnight culture was spread on an LB plate supplemented with hemin, and 50 μl of a solution of 2 mg of NTG per ml was spotted on the center of the plates. After overnight incubation, large satellite colonies were picked and checked for hemin-dependent growth.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ALA, 5-aminolevulinic acid; proto IX, protoporphyrin IX; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.
*To whom reprint requests should be addressed.

Table 1. Bacterial strains

Strain	Relevant genotype or phenotype	Source
CA274		Ref. 4
VS101	<i>visA</i>	Ref. 2
VS200	$\Delta visA$	This work
VS201	$\Delta visA hemA$	This work
VS206	$\Delta visA hemA$	This work
VS215	$\Delta visA hemA$	This work
VS217	$\Delta visA hemA$	This work
VS247	$\Delta visA hemA$	This work
VS202	$\Delta visA$ light resistant (<i>hemB</i> ?)	This work
VS203	$\Delta visA$ light resistant (<i>hemB</i> ?)	This work
VS216	$\Delta visA$ light resistant (<i>hemB</i> ?)	This work
VS207	$\Delta visA$ light resistant (<i>hemC</i> or <i>hemD</i> ?)	This work
VS281	$\Delta visA hem^{P*}$	This work
QC722	$\Delta sodA::lacZ C_m^R$	Ref. 5
VS111	<i>visA</i> $\Delta sodA::lacZ$	P1(QC722) $\times$ VS101
VS011	$\Delta sodA::lacZ$	P1(QC722) $\times$ CA274

Other genotypes are $F^- \Delta lac4169 rpsL$ for QC722 and $HfrC lac_{am}$ for all other strains.

*Hemin permeable.

Assays. Catalase was assayed qualitatively by allowing drops of 30% (vol/vol) H_2O_2 to fall on a colony or a lawn of bacteria and observing the evolution of oxygen gas. For the β -galactosidase assay, 1/1000th volume of an overnight culture was inoculated into LB broth and incubated for 4 hr in complete darkness. Then, after transfer of the cultures to various illumination conditions or to the presence of drug for 45 min, the amount of β -galactosidase was determined as described by Miller (6).

RESULTS

Phenotypes of the *visA* Deletion Mutant. If *visA* truly encodes ferrochelatase, loss of the *visA* function should lead to the loss of activity of enzymes that require heme as a prosthetic group, such as the cytochromes and catalases.

FIG. 1. (A) Photosensitivity of the $\Delta visA$ mutant. Each strain was cultured overnight in the dark with shaking and was inoculated into 5 ml of LB medium (at a final concentration of 2×10^6 bacteria per ml) in plastic Petri dishes. The bacterial cultures were first kept in the dark for 1 hr and then transferred to the light (≈ 7500 lux, at a position 15 cm below two fluorescent lamps, Hitachi Sunlight FL40SSD/37-G, 40 W). At intervals, samples of the illuminated bacteria were withdrawn and plated on LB plates at appropriate dilutions. The plates were incubated in the dark for counting of the colonies. (B) Growth of the $\Delta visA$ mutant. Cultures of the various strains were diluted with LB medium supplemented with glucose and incubated in the dark. At intervals, aliquots of the bacterial culture were plated on LB plates at appropriate dilutions. The plates were incubated in the dark for 2 days before counting the colonies. $\times$, CA274 (wild type); $\blacksquare$, VS200 ($\Delta visA$); $\bullet$, VS101 (*visA*).

However, all of our *visA* mutants reported previously (2) were able to grow well with succinate as a carbon source, suggesting that they might have only partially lost the function of the *visA* product and may retain residual amounts of cytochromes and other hemoproteins. Therefore, we attempted to delete a portion of the *visA* gene on the chromosome, as described in *Materials and Methods*. The resultant strain (VS200) was more sensitive to light than the mutant strain VS101 (Fig. 1A). As expected, VS200 was unable to grow on succinate, and even in rich medium it grew very poorly (doubling time, ≈ 80 min) (Fig. 1B). VS200 also showed no catalase activity, although the point mutant VS101 did show detectable activity (data not shown).

Photosensitivity of *visA* Mutants Is not Caused by the Lack of Heme in the Cell. Because wild-type *E. coli* does not allow heme molecules to penetrate the cell membrane (14), a requirement for heme could not be tested by the addition of heme to the medium. Some *E. coli* mutants can, however, take up and utilize heme, in the form of hemin, when it is supplied in the medium (15). We isolated hemin-permeable mutants from VS200. The growth of one such mutant (VS281) was accelerated with increases in the concentration of hemin in the medium and was restored to the wild-type rate by addition of hemin at $10 \mu\text{g/ml}$ (Fig. 2). We examined the photosensitivity of this mutant with and without hemin in the medium, and in both cases, the mutant bacteria were photosensitive (Fig. 3). Therefore, we concluded that the photosensitivity was not caused by an inability to produce heme.

Isolation and Characterization of Revertants (Photoresistant Mutants) from the *visA*-Deleted Strain. To examine the mechanism of photosensitivity, we attempted to isolate photoresistant mutants from the *visA*-deleted strain VS200 after mutagenesis with NTG. Since a part of the *visA* gene was deleted in VS200, no true revertants of *visA* were expected; the revertants were expected to be double mutants in the *visA* gene and in related genes. In total, 73 independent revertants were selected and analyzed. All of them grew at about the same rate as the parental strain VS200, but none grew as rapidly as the wild-type strain CA274. They showed no catalase activity (data not shown). Complementation of the

FIG. 2. Hemin-dependent growth of a heme-permeable mutant. Cultures of each strain were diluted to an OD₆₆₀ of 0.01 with LB broth supplemented with the indicated concentrations of hemin. After 6 hr of incubation, the OD₆₆₀ was determined. ●, CA274 (wild type); ■, VS281 ($\Delta visA$ heme permeable); ▲, VS200 ($\Delta visA$). The difference between VS281 and VS200 without hemin may be due to the state of the starting culture, because VS200 did not grow as vigorously as other strains, and apparently its growth potential was exhausted prior to the stationary phase.

revertants with $\lambda visA$, which contained the entire *visA* gene, was examined. As shown in Fig. 4A, none of the revertants restored the normal growth rate, in spite of positive complementation of the parental strain. From these observations, we concluded that, in addition to the $\Delta visA$ mutation, these

FIG. 3. Photosensitivity of the hemin-permeable $\Delta visA$ mutant when supplemented with hemin. Overnight cultures of each strain were streaked on LB plates and incubated in the light or in the dark; 24 hr later, the plates were photographed. (A) Plates were supplemented with 10 μ g of hemin per ml. (B) No additions were made to the plates. L, light; D, dark. All the strains are described in Table 1.

revertant strains had second mutations in other genes necessary for respiration.

Identification of *hem* Mutants Among the Revertants. Given that the above-mentioned revertants no longer formed the red-brown colonies characteristic of severely defective *hemH* mutants (2, 16), we hypothesized that they might be defective in the heme synthetic pathway at a step before that catalyzed by the product of *hemH*. To confirm this possibility, complementation of the respiratory defect of the $\lambda visA$ lysogens was again examined, but this time the plates were supplemented with ALA, a specific intermediate in the synthesis of heme (17, 18). If a mutant had a blockage in the biosynthesis of heme at the step before formation of ALA, the $\lambda visA$ lysogen of the revertant should have restored respiratory ability, forming larger colonies. As shown in Fig. 4B, 48 out of 73 revertants showed restoration of the wild-

FIG. 4. Complementation of photoresistant revertants with λ clones of the *visA* and *hem* genes. Lysates of λ phages ($>10^9$ plaque-forming units per ml) were streaked vertically on LB plates, and then cultures of each strain were cross-streaked horizontally over the lysate. Each plate was incubated for 2 days in the dark. If bacteria regain respiratory ability when lysogenized by the phage(s), larger colonies should appear after the cross. Only nine of the mutants are shown in this figure. All the strains are described in Table 1. (A) Lysate of $\lambda visA$ was used. (B) Lysate of $\lambda visA$ was used, but the plate was supplemented with ALA. (C) A mixture of $\lambda visA$ and each of the λ clones of *hemA*, *hemB*, and *hemC-D* was used. Dashed lines with arrowheads indicate the direction in which cultures or lysates were streaked.

Table 2. Growth of the *visA* mutant under anaerobic conditions

Conditions	OD ₆₆₀			
	LB	M9	NO ₃	Fumarate
+ O ₂ , light	0.03/2.37	0.03/1.49	0.00/1.28	0.01/1.22
- O ₂ , light	0.93/0.60	0.75/0.56	0.49/0.54	0.29/0.20
+ O ₂ , dark	2.10/2.40	1.12/1.48	1.19/1.52	1.35/1.53
- O ₂ , dark	0.51/0.66	0.53/0.63	0.51/0.74	0.46/0.56

Overnight cultures of VS101 and CA274 were inoculated into 5 ml of LB medium, M9 medium, fumarate minimal medium, or NO₃⁻ minimal medium in a 10-ml test tube. The minimal media were supplemented with 0.1% Casamino acids and 20 mg of tryptophan per ml. For anaerobic conditions, tubes were tightly capped with rubber stoppers, and the air inside was replaced by N₂ gas, bubbled through the needle of a syringe. After overnight incubation, the OD₆₆₀ was determined. The values given are as the (OD₆₆₀ of VS101)/(OD₆₆₀ of CA274). + O₂, aerobic conditions; - O₂, anaerobic conditions. Light, tubes were illuminated at 7500 lux; dark, tubes were kept in the dark.

type growth rate after they were crossed with a λ *visA* lysate. Thus, it was evident that the second sites of mutation in these 48 revertants lay in the biosynthesis of heme that precedes the formation of ALA. Next, since the DNA sequences of some of the *hem* genes have been reported (15, 19, 20), we searched for clones in which such genes were located by comparing the genetic maps and the restriction maps in Kohara's ordered λ library (10). We found that the *hemA* and the *hemB* genes and the *hemC hemD* operon were carried on clones 4D10, 8F10, and 12G1, respectively. Each of these three λ clones was lysogenized along with λ *visA*, and the complementation of the respiratory defect was examined. Of the 48 revertants that showed ALA-dependent growth in the previous experiment, 46 exhibited restoration of normal growth when lysogenized with phage clone 4D10. These results were also confirmed by complementation with plasmid pHA1, in which only the *hemA* gene was cloned (data not shown). Thus, we conclude that the sites of the second mutation are located in the *hemA* gene. In the same way, revertants 16 and 3 showed restored respiratory ability when lysogenized with phage clones 8F10 and 12G1, respectively, suggesting that in these cases the sites of the second mutation were in the *hemB* gene and *hemC hemD* operon (Fig. 4C).

Addition of ALA Caused Photosensitivity of the *hemA* Revertant. The fact that the second mutation in the *hem* genes eliminated the photosensitivity of the Δ *visA* mutant might indicate that interruption of the heme biosynthetic pathway at proto IX is critical. We confirmed this possibility by adding ALA to the medium, bypassing the *hemA* function. The Δ *visA* revertants harboring a second mutation in the *hemA* gene again displayed photosensitivity when ALA at 100 μ g/ml was added to the plates, whereas the revertants in the

other *hem* genes did not (data not shown). From these observations, we can conclude that a genetic block at the step from proto IX to heme is critical to the photosensitivity.

Molecular Oxygen Is Required for Photosensitivity. It has been reported that various kinds of active oxygen are formed from molecular oxygen by the photochemical reaction of proto IX. If this reaction is the cause of the light-sensitive phenotype of the *visA* mutant, mutant cells should not show photosensitivity when they are deprived of oxygen. We examined the growth of the *visA* mutant under various conditions. As shown in Table 2, the *visA* mutant can also grow in the light when oxygen is absent, clearly supporting the above possibility.

Induction of Genes Inducible by the Active Oxygen. Among the genes that respond to the stress caused by active oxygen, the regulation of *sodA*, *nfo*, and *katG* has been extensively studied (21–24). The former two genes are induced by the superoxide radical (O₂⁻), and the latter is induced by hydrogen peroxide (H₂O₂). We investigated the induction of these three genes by means of gene fusions to *lacZ*. VS101 and CA274 were transformed with pHI201, which carries the *nfo*'-'*lacZ* fusion, or with pKT1033, which carries the *katG*'-'*lacZ* fusion. The transformants were used for the assay of inducible β -galactosidase activity. In addition, the *sodA*'-'*lacZ* fusion gene in strain QC772 was transduced by phage P1 to VS101 and CA274, and the resultant strains, VS111 and VS011, were also examined. Plasmid pUR290 carrying the intact *lacZ* gene was used to transform VS101 and CA274, and these strains were used as controls. As shown in Table 3, the *visA* mutants showed a several-fold induction of β -galactosidase activity in the case of the *sodA* and *nfo* fusions, even in the dark. Compared with the extent of induction by their chemical inducer, however, no significant induction by light was observed in any strain.

DISCUSSION

From the following two pieces of evidence, it is clear that the product of the *visA* gene participates in the biosynthesis of heme. First, the bacterial cells lost the ability to grow on a nonfermentative carbon source when the product of the *visA* gene was absent. Second, in the hemin-permeable derivative of the Δ *visA* mutant, the respiratory deficiency was rescued by the addition of hemin to the medium. Moreover, from our previous finding that *visA* maps very close to *hemH* (the structural gene for ferrochelatase) and that its product exhibits 28% amino acid identity to the ferrochelatase from yeast (2), it was suggested that the *visA* gene encodes the ferrochelatase and is the same gene as *hemH*.

Because the addition of hemin to the medium did not cure photosensitivity even in the hemin-permeable strain, the lack of heme in the cell cannot explain the photosensitivity. We

Table 3. Induction of genes inducible by active oxygen

Fused gene	State of <i>visA</i> [†]	β -Galactosidase activity*				
		Dark	Dark + inducer [‡]	500 lux	2000 lux	5000 lux
Unfused <i>lacZ</i>	+	100	ND	ND	105	ND
	-	124	ND	ND	119	ND
<i>sodA</i>	+	100	260	102	140	116
	-	300	541	282	318	282
<i>nfo</i>	+	100	201	75	95	96
	-	235	439	284	257	253
<i>katG</i>	+	100	379	96	102	113
	-	124	509	127	127	163

ND, not determined.

*Units were adjusted for each fusion such that the dark control (the *visA*⁺ strain) gave a value of 100. Assays were performed as described in *Materials and Methods*.

[†]+, CA274; -, VS101.

[‡]The inducer for *lacZ*, *sodA*, and *nfo* was 10 μ M paraquat. The inducer for *katG* was 40 μ M H₂O₂.

isolated 73 independent photoresistant revertants from the $\Delta visA$ strain. Every one exhibited the respiratory defect, even when the *visA* gene was supplied by the *visA*⁺ transducing phage. Therefore, the second-site mutations, which suppressed the photosensitivity, seemed to be in genes involved in respiration. In fact, at least 67 of them proved to cause blockage at other steps in the heme biosynthetic pathway (i.e., before the formation of proto IX). In the revertants with the *hemA* mutation, in which biosynthesis of heme was blocked before the synthesis of ALA, the photosensitivity was again observed when ALA was supplied in the medium. These observations support the conclusion that the lack of heme does not cause photosensitivity, but rather that the blockage of the biosynthetic flow from proto IX to heme is responsible for the photosensitivity. By analyzing more and as yet unidentified revertants, we should be able to find other mutants in the genes for the biosynthesis of heme that have not yet been cloned, and we may also find other genes involved in the photosensitivity.

Cox and Charles (16) reported that *hemH* mutants accumulate proto IX in the cell and, as a result, their colonies are red in color. Colonies of our $\Delta visA$ strain VS200 are also red in color, and this coloration should be due to the accumulation of the same compound. Although the colonies of the *visA* strains VS101–104, which were described previously (2), were not red in color under the usual growth conditions, they were red under some growth conditions [e.g., during incubation at 42°C (2) or after addition of excess ALA to the medium (data not shown)]. We suggest that some proto IX also accumulates in the mutant cells and is the cause of the photosensitivity, as in the case of the disease protoporphyria in humans.

The photosensitivity of the *visA* mutant was not observed under anaerobic conditions. This phenomenon can be interpreted in either of two ways. First, molecular oxygen (O₂) is indispensable for the photosensitivity. Second, under anaerobic conditions less heme is required by the cell and the synthesis of heme is not active enough to generate toxic levels of proto IX. The second possibility seems to be less probable because the *visA* mutant can grow in the light when NO₃⁻ or fumarate, instead of O₂, is used as the terminal electron acceptor. For such anaerobic respiration, cytochromes and other hemoproteins should be required for growth as they are in the case of aerobic respiration (25).

In protoporphyria, some forms of active oxygen are photochemically produced from O₂. In some reports, singlet oxygen (¹O₂) has been proposed as the product (26, 27), but in another report the formation of O₂⁻ was also suggested (28). The requirement of oxygen for the photosensitivity of our mutant also suggests that some kinds of active oxygen are produced in the light. Two enzymatic defense systems that act against oxidative stress are known in *E. coli*. They are activated under the control of products of the *oxiR* and *soxR* genes and are induced in response to stress caused by H₂O₂ and O₂⁻ respectively (29–31). We investigated the induction by light of three genes under the control of these two genes (*katG*, coding for the catalase-peroxidase hydroperoxidase I, under the control of the *oxiR* regulon; and *sodA*, coding for the Mn-superoxide dismutase, and *nfo*, coding for endonuclease IV, both under the control of the *soxR* regulon). When compared with the wild type, the *visA* mutant showed a certain level of overexpression of the *sodA* and *nfo* genes, but no significant induction by light was observed. This observation may indicate that H₂O₂ and O₂⁻ are not evolved as a

result of illumination. We speculate that the toxicity is due mainly to ¹O₂. At present we have no explanation for the induction of the *sodA* and *nfo* genes at the basal level. However, the accumulated proto IX may produce a certain amount of O₂⁻ even in the absence of light.

We thank Dr. S. Yonei (Department of Zoology, Faculty of Science, Kyoto University) for the generous gift of the plasmids and for valuable discussions. This research was supported by a Grant-in-Aid for Scientific Research on Priority Areas for studies of "The Molecular Mechanism of Photoreception" from the Ministry of Education, Science and Culture of Japan.

- Chartrand, P., Tardif, D. & Sásármán, A. (1979) *J. Gen. Microbiol.* **110**, 61–66.
- Miyamoto, K., Nakahigashi, K., Nishimura, K. & Inokuchi, H. (1991) *J. Mol. Biol.* **219**, 393–398.
- Labbe-Bois, R. (1990) *J. Biol. Chem.* **265**, 7278–7283.
- Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., Brenner, S. & Smith, J. D. (1970) *J. Mol. Biol.* **47**, 1–13.
- Carlioz, A. & Touati, D. (1986) *EMBO J.* **5**, 623–630.
- Miller, J. F. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
- Cohen, G. N. & Rickenberg, H. W. (1956) *Ann. Inst. Pasteur Paris* **91**, 693–720.
- Thomas, M., Cameron, J. R. & Davis, R. W. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4579–4583.
- Kohara, Y., Akiyama, K. & Isono, K. (1987) *Cell* **50**, 495–508.
- Rüther, U. & Müller-Hill, B. (1983) *EMBO J.* **2**, 1971–1974.
- Tao, K., Makino, K., Yonei, S., Nakata, A. & Shinagawa, H. (1989) *Mol. Gen. Genet.* **218**, 371–376.
- Guarneros, G. & Echols, H. (1970) *J. Mol. Biol.* **47**, 565–574.
- Sásármán, A., Surdeanu, M., Szégli, G., Horodniceanu, T., Greceanu, V. & Dumitrescu, A. (1968) *J. Bacteriol.* **96**, 570–572.
- Li, J.-M., Umanoff, H., Proenca, R., Russell, C. S. & Cosloy, S. D. (1988) *J. Bacteriol.* **170**, 1021–1025.
- Cox, R. & Charles, H. P. (1973) *J. Bacteriol.* **113**, 122–132.
- Li, J.-M., Brathwaite, O., Cosloy, S. D. & Russell, C. S. (1989) *J. Bacteriol.* **171**, 2547–2552.
- Avissar, Y. J. & Beale, S. I. (1989) *J. Bacteriol.* **171**, 2919–2974.
- Li, J.-M., Russell, C. S. & Cosloy, S. D. (1989) *Gene* **82**, 209–217.
- Sásármán, A., Nepveu, A., Echelard, Y., Dymetriszyn, J., Drolet, M. & Goyer, C. (1987) *J. Bacteriol.* **169**, 4257–4262.
- Touati, D. (1988) *J. Bacteriol.* **170**, 2511–2520.
- Farr, S. B., D'Ari, R. D. & Touati, D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8268–8272.
- Loewen, P. C., Switala, J. & Triggs-Raine, B. L. (1985) *Arch. Biochem. Biophys.* **243**, 144–149.
- Chan, E. & Weiss, B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3189–3193.
- Inglede, W. J. & Poole, R. K. (1984) *Microbiol. Rev.* **48**, 222–271.
- Lamola, A. A., Yamana, T. & Trozzolo, A. M. (1973) *Science* **179**, 1131–1133.
- Hsu, J., Goldstein, B. D. & Harber, L. C. (1971) *Photochem. Photobiol.* **13**, 67–77.
- Buettner, G. R. & Oberley, L. W. (1979) *FEBS Lett.* **98**, 18–20.
- Christman, M. F., Morgan, R. W., Jacobson, F. S. & Ames, B. N. (1985) *Cell* **41**, 753–762.
- Christman, M. F., Storz, G. & Ames, B. N. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3484–3488.
- Greenberg, J. T., Monach, P., Josephy, P. D. & Demple, B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6181–6185.