

## Swarthmore College Works

Biology Faculty Works

Biology

11-1-1995

# Responses To Toxicants Of An Escherichia Coli Strain Carrying A *uspA'*::*lux* Genetic Fusion And An E. Coli Strain Carrying A *grpE'*::*lux* Fusion Are Similar

T. K. Van Dyk

D. R. Smulski

T. R. Reed


S. Belkin

Amy Cheng Vollmer

*Swarthmore College*, [avollme1@swarthmore.edu](mailto:avollme1@swarthmore.edu)

*See next page for additional authors*

Follow this and additional works at: <http://works.swarthmore.edu/fac-biology>

 Part of the [Biology Commons](#), and the [Microbiology Commons](#)

### Recommended Citation

T. K. Van Dyk, D. R. Smulski, T. R. Reed, S. Belkin, Amy Cheng Vollmer, and R. A. LaRossa. (1995). "Responses To Toxicants Of An Escherichia Coli Strain Carrying A *uspA'*::*lux* Genetic Fusion And An E. Coli Strain Carrying A *grpE'*::*lux* Fusion Are Similar". *Applied And Environmental Microbiology*. Volume 61, Issue 11. 4124-4127.

<http://works.swarthmore.edu/fac-biology/391>

This Article is brought to you for free and open access by the Biology at Works. It has been accepted for inclusion in Biology Faculty Works by an authorized administrator of Works. For more information, please contact [myworks@swarthmore.edu](mailto:myworks@swarthmore.edu).

---

**Authors**

T. K. Van Dyk, D. R. Smulski, T. R. Reed, S. Belkin, Amy Cheng Vollmer, and R. A. LaRossa

## Responses to Toxicants of an *Escherichia coli* Strain Carrying a *uspA'*::*lux* Genetic Fusion and an *E. coli* Strain Carrying a *grpE'*::*lux* Fusion Are Similar

TINA K. VAN DYK, DANA R. SMULSKI, TIMOTHY R. REED, SHIMSHON BELKIN,†  
AMY C. VOLLMER,‡ AND ROBERT A. LAROSSA\*

DuPont Company, Central Research and Development,  
Experimental Station, Wilmington, Delaware

Received 23 May 1995/Accepted 11 August 1995

**A transcriptional fusion of the *Escherichia coli* *uspA* promoter to *luxCDABE* was characterized and compared with a heat shock-responsive *grpE'*::*lux* fusion. Similarities in range and rank order of inducing conditions were observed; however, the magnitude of induction was typically greater for the *grpE'*::*lux* fusion strain.**

One approach to environmental monitoring is to detect changes in gene expression patterns induced by adverse conditions. Bacterial strains that increase light production in the presence of specific chemicals have been constructed by using the bacterial bioluminescence genes (*lux*) as reporters of transcriptional responses (2, 3, 12). A complementary approach, not requiring prior knowledge of expected contaminants, uses less-specific stress responses as general indicators of deleterious conditions. For example, a large variety of environmental challenges triggers the heat shock response (4). Accordingly, *Escherichia coli* strains containing the heat shock promoter *grpE*, *dnaK*, or *lon* fused to the *lux* reporter increase bioluminescence in response to many chemicals (13-15).

Monitoring induction of other stress-induced genes may also be useful for general detection of toxicants. Of particular interest is the *E. coli* *uspA* gene, which encodes the universal stress protein A (9). Conditions that limit cell growth, including nutrient starvation and exposure to toxic agents, induce *uspA* transcription (9, 10). A sequence characteristic of a  $\sigma^{70}$ -driven promoter is found upstream of the apparent *uspA* mRNA start site in cultures exposed to carbon starvation (9) and heat shock (10). This promoter has little homology with  $\sigma^{32}$ -activated promoters (17). The regulatory circuit controlling *uspA* induction is unknown; nevertheless, quantitation of this transcriptional response was anticipated to yield a distinct profile, complementary to that of the  $\sigma^{32}$ -controlled heat shock response.

**Plasmids, *E. coli* strains, and bioluminescence quantitation.** *E. coli* TV1061 with plasmid pGrpELux5 has been described (14). The plasmid pUspALux2, containing a fusion of the *uspA* promoter region to the *Vibrio fischeri* *luxCDABE* genes, was constructed as previously described (14), using for PCR amplification the primers 5'-ACTTAAGGATCCCTCCCGATACGCTGCCA-3' and 5'-AGCAGCGAATTCGGCGATGA GAATGTGTTTAT-3', which included, respectively, nucleo-

tides -213 to -197 and +153 to +135 relative to the major start point of *uspA* transcription (9). *E. coli* DE135 consists of host RFM443 (6) with pUspALux2. The primary structures of the promoter-*lux* fusions were verified by sequencing in one direction from plasmid DNA with a primer from the Tn5 region of the parent plasmid pUCD615 (11), 5'-GGGCTAAATCTGTGTTCTCTTCGG-3' (16). Sequences from plasmids pGrpELux5 and pUspALux2 were identical to the promoter region sequences of *grpE* (5) and *uspA* (9), respectively.

The size and orientation of the inserted DNA were verified by PCR amplification of the inserted promoter region with the isolated plasmid DNA as the template and a primer, 5'-CTGTTCATTAATAGGCAT-3', from the *luxI* region of pUCD615, paired with the previously specified upstream primer for *uspA* or the primer 5'-GAAGATTGATGACAA-3' for *grpE*. The observed sizes of the amplification products corresponded with those predicted for the *grpE* and *uspA* fusions, 1,056 and 1,181 bp, respectively. This technique verified the initial constructs and confirmed the identity of the *uspA'*::*lux* and the *grpE'*::*lux* fusion strains from cultures used in independent induction experiments.

Light production was measured over an 80-min interval at 26°C with cells grown in LB medium (7) as previously described (14). Specific induction units (SIU), representing the increase in bioluminescence due to the presence of stress, normalized to the cells in the assay, were calculated as described previously (15).

**Induction of the *uspA'*::*lux* fusion.** The *E. coli* strain containing the plasmid-borne operon fusion linking the *uspA* promoter to the *luxCDABE* reporter was characterized initially by challenge with several classes of chemical stressing agents. Figure 1 shows the kinetics of bioluminescence changes induced by various concentrations of *n*-propanol, phenol, copper sulfate, and sodium propionate. The presence of each of these compounds resulted in increased light output, with the increase being dependent on inducer concentration. This concentration dependence was bimodal in some cases (Fig. 1A, *n*-propanol, and Fig. 1B, phenol); concentrations below and above the optimal inducing concentration resulted in lower levels of light production. The decrease in bioluminescence at a high concentration of a stressing agent has been observed with other *lux* fusions (1, 3, 12, 14, 15). This decrease is likely due to the toxicity associated with inactivating Lux proteins or reducing metabolite levels, because light production requires

\* Corresponding author. Mailing address: DuPont Company, Central Research and Development, P.O. Box 80173, Wilmington, DE 19880-0173. Phone: (302) 695-9264. Fax: (302) 695-9183. Electronic mail address: larossa@esvax.dnet.dupont.com.

† Present address: Ben-Gurion University of the Negev, Sede-Boqer 84 990, Israel.

‡ Present address: Swarthmore College, Swarthmore, PA 19081-1397.

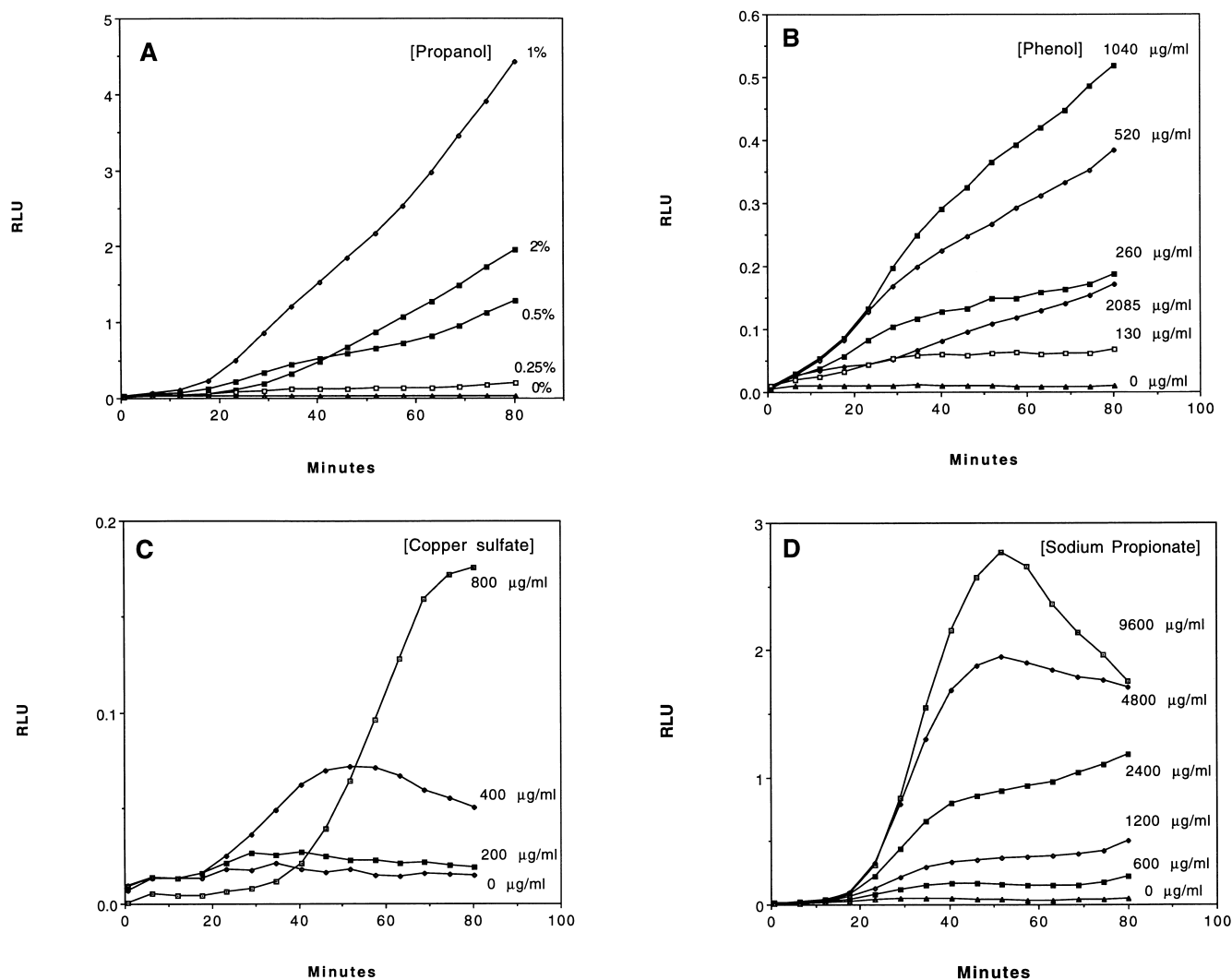


FIG. 1. Induction of increased bioluminescence from an *E. coli* strain containing a *uspA':lux* fusion. *E. coli* DE135 was challenged with *n*-propanol (A), phenol (B), copper sulfate (C), and sodium propionate (D) at the indicated concentrations. The time course of bioluminescence development at 26°C is shown. RLU, relative light units.

active cell metabolism. Thus, the concentrations that yielded maximal induction were sublethal.

**Comparison of responses of strains carrying *uspA':lux* and *grpE':lux* fusions.** The induction of the *uspA':lux* fusion by a variety of stresses was reminiscent of the heat shock-responsive *grpE':lux* fusion (14). A direct comparison of *E. coli* strains containing these two fusions was made by using 25 stress conditions; the results are summarized in Table 1. The inducing agents are listed in order of maximum SIU of the *uspA':lux* fusion strain. The rank order of the inducer strength was very similar to that observed with the *grpE':lux* fusion strain. For both, alcohols tended to be the strongest inducers. Another similarity between the two strains was the range of compounds that resulted in bioluminescence induction. Extremely weak inducers (those with SIU of <0.1) and noninducers of both strains included mitomycin C, Triton X-100, sodium dodecyl sulfate, puromycin, and mercury(II) chloride. One exception to the similarity of responses was that UV irradiation, which was a very poor inducer of the *uspA':lux* fusion strain, resulted in measurable induction of the *grpE':lux* fusion strain. The degree of induction (SIU) was, in most cases, greater for strains

containing the *grpE':lux* fusion than for those containing the *uspA':lux* fusion. When the lowest tested concentration that yielded a twofold increase in bioluminescence over the untreated control was used as an arbitrary estimation of the detection limit, the *grpE':lux* fusion strain typically detected concentrations equal to or somewhat lower than those detected by the *uspA':lux* fusion strain.

The many similar characteristics of stress induction between *uspA':lux* and *grpE':lux* fusion strains were unexpected, given the differing regulatory circuits controlling their expression. Alterations in plasmid copy number are unlikely to be responsible for the increases in bioluminescence because of the relatively short time of the stress induction experiment (one doubling time or less). Indeed, the plasmid content of cells containing a closely related plasmid having a different stress promoter is unaltered by three stresses (15). Several other explanations are possible for the similarities of the two fusions; further experimentation is required to distinguish among them.

The utility of the universal stress protein response of *E. coli* for detecting many environmental stresses was shown by using a fusion of the *uspA* promoter to the *V. fischeri luxCDABE*

TABLE 1. Comparison of *uspA'::lux* and *grpE'::lux* inductions

Inducer <sup>a</sup>	<i>uspA'::lux</i>			<i>grpE'::lux</i>		
	Concn for maximum induction	SIU	Minimum concn for twofold increase	Concn for maximum induction	SIU	Minimum concn for twofold increase
Ethanol	2% (vol/vol)	7.4	0.25% (vol/vol)	4% (vol/vol)	50.0	0.12% (vol/vol) <sup>b</sup>
Isopropanol	2% (vol/vol)	7.0	0.12% (vol/vol) <sup>b</sup>	2% (vol/vol)	27.6	0.12% (vol/vol) <sup>b</sup>
Propanol	1% (vol/vol)	6.8	0.12% (vol/vol) <sup>b</sup>	1% (vol/vol)	20.2	0.12% (vol/vol) <sup>b</sup>
Butanol	0.5% (vol/vol)	3.1	0.12% (vol/vol) <sup>b</sup>	1% (vol/vol)	6.5	0.12% (vol/vol) <sup>b</sup>
Sodium propionate	9,600 µg/ml	2.6	300 µg/ml	9,600 µg/ml	4.7	600 µg/ml
Formamide	4% (vol/vol)	1.8	0.062% (vol/vol) <sup>b</sup>	4% (vol/vol)	1.4	0.062% (vol/vol) <sup>b</sup>
4-Nitrophenol	50 µg/ml	1.2	6.2 µg/ml	50 µg/ml	5.2	3.1 µg/ml
Phenol	1,040 µg/ml	0.55	65 µg/ml <sup>b</sup>	1,040 µg/ml	3.8	65 µg/ml <sup>b</sup>
Sodium acetate	8,200 µg/ml	0.36	510 µg/ml	4,100 µg/ml	0.64	510 µg/ml
NaCl	20,000 µg/ml	0.29	5,000 µg/ml	20,000 µg/ml	0.61	2,500 µg/ml
CuSO <sub>4</sub>	800 µg/ml	0.17	400 µg/ml	800 µg/ml	1.6	100 µg/ml
Pentachlorophenol	37.5 µg/ml	0.17	4.7 µg/ml	37.5 µg/ml	2.1	4.7 µg/ml
Methanol	4% (vol/vol)	0.17	0.5% (vol/vol)	4% (vol/vol)	9.8	0.25% (vol/vol)
Hydrogen peroxide	50 µg/ml	0.13	50 µg/ml	50 µg/ml	1.5	12 µg/ml
Naladixic acid	7.5 µg/ml	0.10	3.8 µg/ml	15 µg/ml	0.95	1.9 µg/ml <sup>b</sup>
Menadione	2,000 µg/ml	0.07	ND <sup>c</sup>	2,000 µg/ml	1.7	500 µg/ml
2,4-Dinitrophenol	125 µg/ml	0.04	63 µg/ml	63 µg/ml	0.50	63 µg/ml
Cadmium chloride	12 µg/ml	0.04	ND	23 µg/ml	0.20	23 µg/ml
Methyl viologen	125 µg/ml	0.02	ND	125 µg/ml	0.17	62.5 µg/ml
UV irradiation	49,900 µJ/cm <sup>2</sup>	0.01	ND	49,900 µJ/cm <sup>2</sup>	0.29	1,800 µJ/cm <sup>2</sup>
Mitomycin C	1.25 µg/ml	0.01	ND	1.25 µg/ml	0.00	ND
Triton X-100	0.06% (vol/vol)	0.01	ND	0.06% (vol/vol)	0.01	ND
Puromycin	No induction	ND	ND	200 µg/ml	0.02	ND
Sodium dodecyl sulfate	No induction	ND	ND	No induction	ND	ND
HgCl <sub>2</sub>	No induction	ND	ND	No induction	ND	ND

<sup>a</sup> Stock solutions in water were diluted into LB medium (10) for cadmium chloride (100 mM stock), copper(II) sulfate pentahydrate (250 mM stock), 2,4-dinitrophenol (2-mg/ml stock), mercury(II) chloride (100 mM stock), and mitomycin C (1-mg/ml stock). Naladixic acid was diluted into LB medium from a 100-mg/ml stock solution in 1 N NaOH. Pentachlorophenol was diluted from a 100-mg/ml solution in ethanol, and 4-nitrophenol was diluted from a 400-mg/ml solution in methanol; the final concentrations of ethanol and methanol after the dilution did not induce a stress response from these promoters. All other compounds were added directly to the LB medium. For assessing induction by UV irradiation, cells in LB medium were irradiated at various doses with a Stratilinker UV Crosslinker (Stratagene); 100-µl samples were transferred to microtiter plates for measurement of bioluminescence.

<sup>b</sup> Lowest concentration tested.

<sup>c</sup> ND, not detected; none of the concentrations tested resulted in induction values more than twofold higher than values for the uninduced control.

reporter genes. However, a side-by-side comparison with another generally responsive genetic fusion, *grpE'::lux*, did not reveal an advantage of the *uspA'::lux* fusion. The strain containing the heat shock promoter fusion had stronger induction responses, better detection limits, and a broader range of detectable stresses. Furthermore, the heat shock response is universally present in cells (8); hence, measurement of this response in bacteria may have relevance to all organisms. The extent of *uspA* conservation is not presently known, although DNA-DNA hybridization experiments indicate that some members of the family *Enterobacteriaceae* contain an analog of the *uspA* gene (16). Nevertheless, monitoring the universal stress protein response should not be discounted because changes to maximize the sensitivity of these tests, such as the use of permeability mutants (14) or alteration of the growth and test medium, may differentially effect the utility of the *grpE'::lux* and *uspA'::lux* fusions.

We thank Mary Bailey for synthesis of oligonucleotides.

#### REFERENCES

- Belkin, S., A. C. Vollmer, T. K. Van Dyk, D. R. Smulski, T. R. Reed, and R. A. LaRossa. 1994. Oxidative and DNA damaging agents induce luminescence in *E. coli* harboring *lux* fusions to stress promoters, p. 509–512. In A. K. Campbell, L. J. Kricka, and P. E. Stanley (ed.), *Bioluminescence and chemiluminescence: fundamentals and applied aspects*. John Wiley & Sons Ltd., Chichester, England.
- Burlage, R. S., G. S. Saylor, and F. Larimer. 1990. Monitoring of naphthalene catabolism by bioluminescence with *nah-lux* transcriptional fusions. *J. Bacteriol.* **172**:4749–4757.
- Corbisier, P., G. Ji, G. Nuyts, M. Mergeay, and S. Silver. 1993. *luxAB* gene fusions with the arsenic and cadmium resistance operons of *Staphylococcus aureus* plasmid pI258. *FEMS Microbiol. Lett.* **110**:231–238.
- Georgopoulos, C., K. Liberek, M. Zylitz, and D. Ang. 1994. Properties of the heat shock proteins of *Escherichia coli* and the autoregulation of the heat shock response, p. 209–250. In R. I. Mortimoto, A. Tissieres, and C. Georgopoulos (ed.), *The biology of heat shock proteins and molecular chaperones*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Lipinska, B., J. King, D. Ang, and C. Georgopoulos. 1988. Sequence analysis and transcriptional regulation of the *Escherichia coli* *grpE* gene, encoding a heat shock protein. *Nucleic Acids Res.* **16**:7545–7562.
- Menzel, R. 1989. A microtiter plate-based system for the semiautomated growth and assay of bacterial cells for β-galactosidase activity. *Anal. Biochem.* **181**:40–50.
- Miller, J. H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Mortimoto, R. I., A. Tissieres, and C. Georgopoulos. 1994. Progress and perspective on the biology of heat shock proteins and molecular chaperones, p. 1–30. In R. I. Mortimoto, A. Tissieres, and C. Georgopoulos (ed.), *The biology of heat shock proteins and molecular chaperones*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Nyström, T., and F. C. Neidhardt. 1992. Cloning, mapping and nucleotide sequencing of a gene encoding a universal stress protein in *Escherichia coli*. *Mol. Microbiol.* **6**:3187–3198.
- Nyström, T., and F. C. Neidhardt. 1994. Expression and role of the universal stress protein, UspA, of *Escherichia coli* during growth arrest. *Mol. Microbiol.* **11**:537–544.
- Rogowsky, P. M., T. J. Close, J. A. Chimera, J. J. Shaw, and C. I. Kado. 1987. Regulation of the *vir* genes of *Agrobacterium tumefaciens* plasmid pTiC58. *J. Bacteriol.* **169**:5101–5112.
- Selifonova, O., R. Burlage, and T. Barkay. 1993. Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. *Appl. Environ. Microbiol.* **59**:3083–3090.
- Van Dyk, T. K., S. Belkin, A. C. Vollmer, D. R. Smulski, T. R. Reed, and R. A. LaRossa. 1994. Fusions of *Vibrio fischeri* *lux* genes to *Escherichia coli* stress

- promoters: detection of environmental stress, p. 147–150. *In* A. K. Campbell, L. J. Kricka, and P. E. Stanley (ed.), *Bioluminescence and chemiluminescence: fundamentals and applied aspects*. John Wiley and Sons Ltd., Chichester, England.
14. **Van Dyk, T. K., W. R. Majarian, K. B. Konstantinov, R. M. Young, P. S. Dhurjati, and R. LaRossa.** 1994. Rapid and sensitive pollutant detection by induction of heat shock gene-bioluminescence gene fusions. *Appl. Environ. Microbiol.* **60**:1414–1420.
  15. **Van Dyk, T. K., T. R. Reed, A. C. Vollmer, and R. A. LaRossa.** Synergistic induction of the heat shock response in *Escherichia coli* by simultaneous treatment with chemical inducers. *J. Bacteriol.* **177**:6001–6004.
  16. **Vollmer, A. C.** Unpublished data.
  17. **Yura, T., H. Nagai, and H. Mori.** 1993. Regulation of the heat-shock response in bacteria. *Annu. Rev. Microbiol.* **47**:321–350.