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MEMBRANE DAMAGE AND RECOVERY ASSOCIATED WITH GROWTH DELAY INDUCED BY NEAR-UV RADIATION IN *Escherichia coli* K-12

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Abstract—The growth delay induced by near-UV radiation has been largely attributed to injured tRNA's and to the stringent response. We report an associated membrane perturbation whose recovery determines substantial modifications in the behavior of log phase *Escherichia coli* K-12 exposed to sublethal doses of near-UV radiation (366 nm). When incubated at 37°C in plain nutrient broth, cells suffered a growth delay of about 100 min with parallel inhibition of several membrane functions. Conversely, when grown in conditions known to influence membrane activities, these were slightly inhibited and the growth delay lasted about 50 min. All the above conditions triggered the stringent response, characterized by an equivalent post-irradiation burst of intracellular guanosine 5′3′ tetra and pentaphosphate and by a similar decay rate of the nucleotides accumulated at time 0 of the growth lag. According to our data the polyphosphates' half decay time in irradiated cells remains practically constant and close to 15 min. But, while cells from unsupplemented broth at 37°C resumed normal growth in around 100 min those with recovered membranes were rescued from growth inhibition in about one half of that time.

INTRODUCTION

Exposure to sublethal fluences of near-UV radiation (300–400 nm), induces in growing *E. coli* a transient inhibition of growth (Jagger *et al.*, 1964) related to the photochemical cross-linking of 4-thiouridine, a rare base of specific tRNA's (Thomas and Favre, 1975; Ramabhadran and Jagger, 1976). In a recent review (Favre *et al.*, 1985), concluded that the photodamage led to accumulation of uncharged $tRNA^{Phe}$ and $tRNA^{Pro}$ followed by a slow down in protein synthesis. In $relA^+$ strains the growth delay is amplified by the stringent response, which triggers an overproduction of the nucleotides guanosine 5′3′ tetra and pentaphosphates (ppGpp and pppGpp)† with subsequent restriction on the synthesis of new functional tRNA's (Thiam and Favre, 1984). The phenomenon was better understood when mutants with lowered growth delay, deficient in 4-thiouridine or in the stringent response were isolated (Ramabhadran *et al.*, 1976; Thomas and Favre, 1980). More recently Blanchetot *et al.* (1984), later supported by Hajnsdorf and Fabre (1986), proposed a tRNA repair hypothesis implying the scission of cross-linked 4-thiouridine-cytosine in photodamaged tRNA's, followed by reinsertion of the corresponding nucleoside in their respective positions.

With the purpose to clarify further the phenomenon, and based on reports suggesting a close relationship between membrane functions and growth delay (Taber *et al.*, 1978; Kubitschek and Doyle, 1981), we analyzed the post-irradiation uptake of [^{14}C]alanine and the specific activity of succinic dehydrogenase (SDH), as probes of membrane functions, in *E. coli* K-12 exposed to a total fluence of 1.2×10^5 J/m^2 of near-UV radiation. When cells were incubated at 37°C in either minimal or nutrient broth, we found that both membrane functions were strongly inhibited in parallel with a 90 min inhibition of growth, and that they were accompanied by intracellular accumulation of high concentrations of [^{32}P]-labeled ppGpp and pppGpp. In this case, membrane functions and nucleotide values recovered to basal levels in coincidence with the end of the lag induced by near-UV exposure.

On the contrary, when cells were incubated in conditions known to influence membrane activities (like temperature shifts or the presence of either glycerol or sucrose during growth) the growth delay was considerably shortened with membrane-bound enzymes and amino acid transport only slightly depressed. Although intracellular ppGpp and pppGpp accumulated in irradiated cells were degraded with an equivalent half decay time in all the assayed conditions, cells incubated in plain broth at 37°C resumed normal growth later than those grown in the presence of glycerol or sucrose, or when submitted to temperature shifts.

We postulate the occurrence of membrane perturbation(s), induced in *E. coli* K-12 by the exposure to 1.2×10^5 J/m^2 of 366 nm UV-radiation which,

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†*Abbreviations*: NADH, reduced nicotinamide adenine dinucleotide; OD, optical density; ppGpp, 5′3′ guanosine tetraphosphate; pppGpp, 5′3′ guanosine pentaphosphate; SDH, succinic dehydrogenase; tRNA, transfer ribonucleic acid; TYB, tryptone yeast extract broth.

associated with photodamaged tRNA's and the stringent response, originated the transient inhibition of growth observed after the illumination. Changes in culture conditions may determine substantial modifications in the extent of the growth delay, allowing an adequate environment for cells recovering normal functions.

MATERIALS AND METHODS

Bacterial strain. Escherichia coli K-12 (ATCC 15153), a wild type F^+ prototroph originally J. Lederberg K-12 W-3005, was used throughout. *Growth conditions.* Unless otherwise indicated *E. coli* K-12 was incubated with vigorous shaking at either 30, 37 or 43°C, in a tryptone yeast extract broth (TYB), containing: tryptone 10 g, yeast extract 5 g, distilled water 1 ℓ. When required media were supplemented with 200 m*M* glycerol or 300 m*M* sucrose. Optical density (OD) was monitored at 650 nm with a spectrophotometer (Model UV-120-02, Shimadzu Corporation, Tokyo 160, Japan).

Irradiation procedures and growth delay measurements. According to Jagger (1981) the growth delay is represented by a horizontal displacement of the growth curve, plotted as log OD/time, and is followed by a small change in the slope when growth reinitiates. Our growth delay experiments started always with cells taken from an overnight primary culture in TYB at 37°C. Aliquots were diluted to an OD of about 0.100 in required media and incubated at the corresponding temperature until an OD of about 0.800 was reached. The cultures were then centrifuged at 4000 rpm for 10 min, cells were resuspended in physiological saline solution (150 m*M* NaCl), placed in an ice bath at 20 cm from the lamp filter surface and irradiated under constant magnetic stirring. Immediately after illumination cells were spun down, resuspended in prewarmed post-irradiation medium (see text) and the growth inhibition was followed by measuring OD as a function of time. Unirradiated controls were similarly treated except that cells were kept in the dark during the irradiation period.

A high intensity longwave (366 nm) ultraviolet lamp was the radiation source (Model B-100A, Ultraviolet Products, San Gabriel, CA). According to the manufacturer, more than 95% of this lamp UV emission is peaked at 366 nm. The lamp comes equipped with a filter that cuts out 100% of visible light and 40% of 340 nm, the second largest UV peak, equivalent to about 1% of the irradiance at 366 nm. The incident fluence in our experimental conditions, 33.4 W/m^2, was measured with a longwave UV meter (J-221 Blak Ray, UV Products), cross-checked against a 9811-58 Cole-Parmer Radiometer (Cole-Parmer Instruments Co. Chicago, IL). Reading of both dosimeters agreed within a ±5% difference for any given point.

Measurement of ppGpp and pppGpp. To measure *in vivo* levels of ppGpp and pppGpp cells were grown from an OD of 0.100 to 0.800 in media containing 50 μCi/mℓ of carrier-free [^{32}P]orthophosphoric acid. Cells were washed-free of the radioactive nutrient, resuspended in saline solution and exposed to a total near-UV fluence of 1.2×10^5 J/m^2. Immediately after illumination cells were collected by centrifugation and incubated in corresponding prewarmed medium (see text) with no exogenous [^{32}P]. At given times, samples were taken to follow growth delay and to simultaneously measure degradation of intracellular ppGpp and pppGpp by following the procedure of Cashel (1969). Twenty microliters of supernatant after formic acid extraction were run in polyethylenimine cellulose plates kindly provided by Sigma Cooperative Project. Radioactive spots, localized by autoradiography, were scraped off the plates and counted in a Model 4530 TriCarb Liquid Scintillation System (Packard Instruments Co., Downers Grove, IL).

Measurement of SDH activity. To follow SDH post-irradiation activity, cells were grown in medium E (Vogel and Bonner, 1956) composed of citric acid 2 g, K_2HPO_4 10 g, $Na_2(NH_4)PO_4.H_2O$ 3 g, $MgSO_4$ 0.2 g, distilled water 1 ℓ. Medium E was supplemented with 7.5 m*M* sodium succinate and the enzyme was assayed in cell-free extracts by measuring ferricyanide reductase activity, according to reported procedures (Kim and Bragg, 1971), except that NADH was omitted and that 10 m*M* was the ferricyanide concentration used.

Measurement of [^{14}C]alanine uptake. Cells were grown in medium E supplemented with 5 m*M* cold alanine and after near-UV exposure reincubated in fresh medium E containing 70 μCi/mℓ [^{14}C]alanine. At corresponding times aliquots were filtered through Millipore-type membranes and thoroughly washed with 10 m*M* alanine solutions in medium E without carbon sources. Filters were dried, transferred to vials containing 5 mℓ of scintillation fluid and counted as above.

Determination of proteins. The concentration of proteins was determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as a standard.

Chemicals. All reagents were analytical grade. Culture media were from Difco Laboratories (Detroit, MI) and radioactive compounds from Comisión Nacional de Energía Atómica (Buenos Aires, Argentina) or from NEN Products (E. I. du Pont de Nemours, Boston, MA).

RESULTS AND DISCUSSION

The transient block in cell growth produced by sublethal doses of near-UV radiation in *E. coli* depends not only on the total dose, but for exponentially growing cells, also on the fluence rate (Favre, 1980). We found that in our experimental conditions the inhibition of growth produced by exposure to a fluence of 1.2×10^5 J/m^2 of near-UV radiation was proportionally reduced when fluences delivered were lowered (Fig. 1). Cells were irradiated in saline solutions at a fluence rate of 33.4 W/m^2. The lamp was free from contaminant UVC and plate counts performed during the illumination and the lag

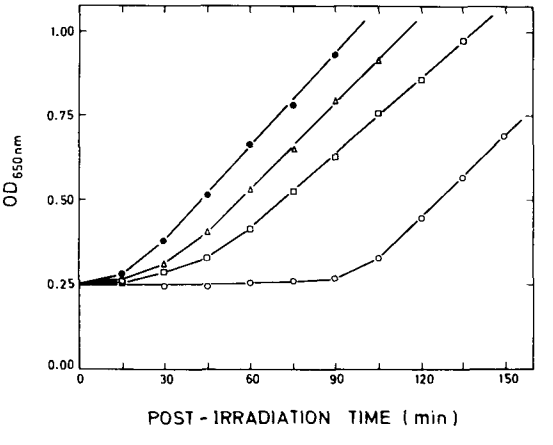

Figure 1. Growth delay induced by near-UV radiation. Cells grown in TYB at 37°C from an OD of 0.100 to 0.800 were centrifuged, resuspended in saline solution and irradiated as described in Materials and Methods. Fluences in J/m^2: (△) 3×10^4; (□) 6×10^4; (○) 1.2×10^5. (●) unirradiated controls. Growth delay was followed at 37°C in TYB.

showed no decrease in cells viability (data not shown). In unpublished observations we found that the extent of the growth delay could be modified by temperature shifts and also by the addition of 400 m*M* ethanol to pre-irradiation medium, suggesting that the environment may determine an adaptive response impelling cells to attain the most favorable conditions for growth. On this basis we postulate that *E. coli* K-12 envelope may be a target for near-UV radiation contributing to extend the photodamage produced in tRNA's. Our postulate is inferred from the finding that temperature shifts and other environmental changes, known to influence membrane composition and properties, determine substantial modifications in the extent of the growth delay.

We report the induction of a growth inhibition lasting approximately 100 min when cells are incubated in TYB at 37°C, temperature considered optimum for *E. coli* growth. But, when post-irradiation temperature was shifted to either 43° or 30°C the lag's extent was considerably shortened or enlarged as shown in Fig. 2. It may be seen that the shift-up constituted a more adequate environment for

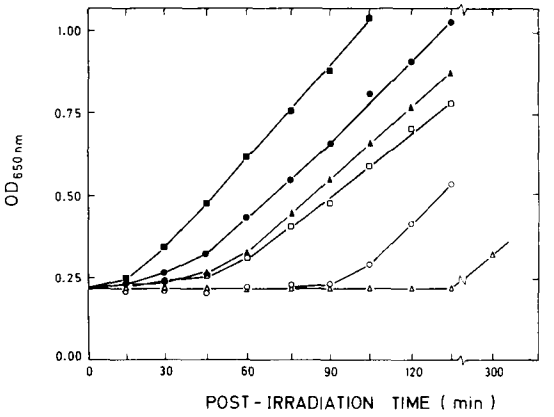

Figure 2. Effect of post-irradiation temperature shifts on the growth delay induced by 1.2×10^5 J/m^2 near-UV radiation. Cells grown in TYB at 37°C were irradiated (see Materials and Methods) and the effect followed in TYB at: (△) 30°; (○) 37° and (□) 43°C. Unirradiated controls [(▲) 30°; (●) 37° and (■) 43°C] were kept in the dark during the irradiation procedure.

recovery, cells resuming normal growth in about 60 min, while the temperature shift-down provided a comparatively less favorable post-irradiation conditions. The responses of membrane composition to environmental perturbations were examined by Thompson (1980) and by Quinn (1981) who stated that membrane's fluidity and chemical composition are subject to significant modifications by many external factors. Hydrophobic interactions may also play an important role in promoting recovery of perturbed membranes, since it was reported that changes in the strength of such interactions may influence *E. coli* growth (Ingram, 1982). Supported by a report of Back *et al.* (1979) demonstrating that

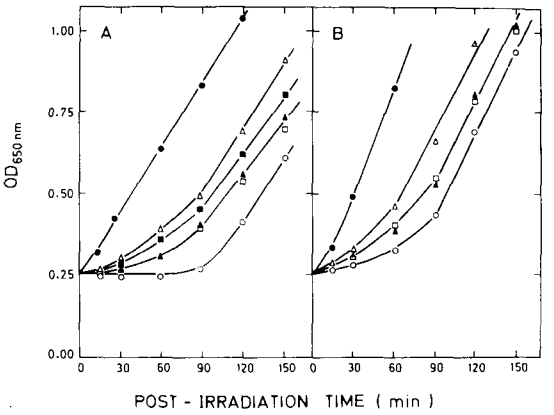

Figure 3. Glycerol and temperature effects on the growth delay induced by 1.2×10^5 J/m^2 near-UV radiation. Cells were grown from an OD of 0.100 to 0.800 at 37°C in TYB containing the following glycerol concentration (m*M*): (○) 0; (□) 25; (▲) 50; (■) 100 and (△) 200 Cells were irradiated in saline solutions (see text), centrifuged and resuspended in unsupplemented TYB and the lag followed at either 37°C (A) or 43°C (B). Unirradiated controls (●) were kept in the dark during the illumination. Analogous results were obtained when medium E was used instead of TYB.

hydrophobic interactions are stronger in glycerol or sucrose solutions, we assayed the effects of these agents in the extent of the growth delay at concentrations given below.

Pre-irradiation growth from an OD of 0.100 to 0.800 in TYB containing 200 m*M* glycerol at 37°C, reduced the post-irradiation lag to about one-third of that obtained in cells grown without the polyol (Fig. 3A). The dependence of glycerol concentration, added to pre-irradiation medium, was studied in the range of molarities reported by Peak *et al.* (1982) to protect *E. coli* B/r against lethal effects of near-UV radiation. In our standard procedures, 200 m*M* appeared as optimum glycerol concentration to improve recovery of K-12 from the illumination (Fig. 3). As stated above, post-irradiation temperature shift-up to 43°C shortened the growth delay of cells previously grown at 37°C in unsupplemented TYB (Fig. 2). On this basis we assayed the effect of combining the protective action of glycerol and the upgrade of post-irradiation temperature. It was found that they are supplementary, since the lag induced by combined action of both factors was significantly shorter after exposure to 1.2×10^5 J/m^2 of near-UV radiation (Fig. 3B).

The protective effect of glycerol against biological actions of near-UV radiation was thoroughly studied by Peak and Peak (1980, 1982) and by Peak *et al.* (1982). They reported that the presence of glycerol during irradiation protects *E. coli* from lethality. To explain the phenomenon they analyzed several possibilities, none of which seem adequate to interpret our results obtained on K-12 grown in the presence of glycerol, but exposed on its absence to sublethal doses of near-UV radiation. We suggest

that glycerol and sucrose (see below) may act by a mechanism similar to that reported for temperature shifts or ethanol addition, as inducers of changes on membrane structure and functions in enterobacteria (Ingram, 1982; Pizarro *et al.*, 1985).

Pre-irradiation growth in the presence of 300 m*M* sucrose exerts an analogous effect to glycerol in the extent of the growth delay. Here again, cells were irradiated in saline solutions and the growth inhibition measured in unsupplemented TYB. As seen in Fig. 4A, cells grown pre-irradiation with the sugar recovered ability to grow normally after the illumination in about 40 min, instead of the 100 min required for controls. Furthermore, when post-irradiation temperature was shifted from 37° to 43°C cells recovered even faster (Fig. 4B). As in the glycerol experiments, there is a moderate influence of sucrose concentration on the reported effect, 300 m*M* appearing as the optimum sucrose amount to stimulate reinitiation of growth (Fig. 4).

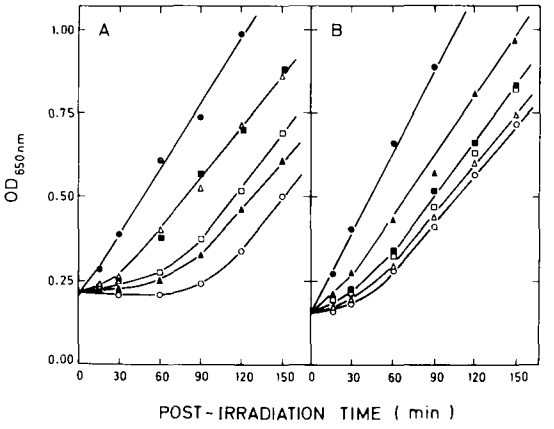

Figure 4. Sucrose and temperature effects on the growth delay induced by 1.2×10^5 J/m^2 near-UV radiation. Cells were grown from an OD of 0.100 to 0.800 at 37°C in TYB containing the following sucrose concentrations (m*M*): (○) 0; (▲) 75; (□) 150; (△) 300 and (■) 400. Cells were irradiated in saline solution (see text), centrifuged, resuspended in unsupplemented TYB and the lag followed at either 37°C (A) or 43°C (B). Unirradiated controls (●) were kept in the dark during the illumination. Analogous results were obtained when medium E was used instead of TYB.

The action of the osmotic strength of a given growth medium on membranes fluidity was reviewed by Quinn (1981) and Broekmann and Steenbakers (1973) reported that a temperature-sensitive *E. coli* K-12 mutant grew at the restrictive temperature in medium supplemented with either 600 m*M* sucrose, 300 m*M* NaCl or 300 m*M* KCl, stating that it is not known whether the effect was due to the osmotic pressure or to the activation of temperature-sensitive enzymes. The sucrose effect reported here seems unrelated with osmotic pressure since we worked at much lower sucrose concentration and because the growth delay in our irradiation conditions were not modified by 300 m*M* NaCl or KCl (data not shown).

Several authors have stated that selective photolesions may be useful in identifying components associated with particular processes or structures (Sharma and Jagger, 1981; Sprott *et al.*, 1976). Therefore, we assumed that if membranes were damaged we should find concomitant alterations in biochemical functions, such as amino acid transport or activity of membrane-bound enzymes. Even more, the environmental conditions which we found as able to modify the growth delay, should also alter the behavior of the affected functions. Based on reports stating that near-UV inhibits amino acid transport (Kubitschek and Doyle, 1981; Peak *et al.*, 1982) we studied [^{14}C]alanine uptake in our experimental conditions. When cells were incubated at 37°C the amino acid uptake ceased immediately after the irradiation, recovering gradually to pre-exposure values. On the contrary, the [^{14}C] uptake was only slightly depressed when cells were submitted to a post-irradiation temperature shift-up (37° to 43°C), or when grown in the presence of either 200 m*M* glycerol or 300 m*M* sucrose (Table 1).

Succinic dehydrogenase (SDH) is a membrane-bound enzyme whose properties were thoroughly studied (Hederstedt and Rutberg, 1981). Based on a report indicating that near-UV inhibits respiration of succinate-linked oxidation in a membrane system (Brodie and Ballantine, 1960), we found that the specific activity of this enzyme — assayed *in vitro* — was arrested during the growth delay occasioned

Table 1. [^{14}C]alanine uptake in *E. coli* K-12 near-UV induced growth delay

		Pre-irradiation supplem. and post-irradiation temp.:			
Min after irradiation	Unirradiated controls	None 37°C	None 43°C	Sucrose 43°C	Glycerol 43°C
0	100	100	100	100	100
15	190	100	140	170	160
30	310	170	260	290	230

Cells grown from an OD of 0.100 to 0.800 at 37°C in medium E + 5 m*M* alanine, with or without 300 m*M* sucrose or 200 m*M* glycerol, were exposed to 1.2×10^5 J/m^2 near-UV, centrifuged and resuspended to an OD of 0.200 in fresh medium E added only with [^{14}C]alanine. They were then incubated to measure the uptake at either 37°C or 43°C (see above). Results are expressed as percentage of [^{14}C]alanine uptake at time zero.

Table 2. SDH activity during near-UV induced growth delay in *E. coli*

Min after irradiation	SDH activity (nmol/mg/h)	
	Unirradiated	Irradiated
0	18.4	2.7
30	9.3	3.3
60	11.6	2.4
120	17.7	6.0

Cells were grown at 37°C in medium E + 7.5 m*M* sodium succinate from an OD of 0.100 to about 0.800, exposed to 1.2×10^5 J/m^2 near-UV radiation, then diluted to an OD of 0.200 and reincubated at the same temperature in fresh medium E + succinate. At given times samples were taken to measure SDH activity in cell free extracts (see Materials and Methods).

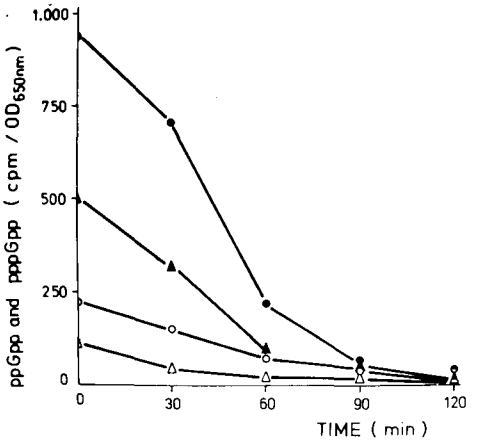

Figure 5. [^{32}P]-labeled ppGpp and pppGpp degradation during growth delay. Cells grown from an OD of 0.100 to 0.800 in TYB containing [^{32}P]orthophosphoric acid were harvested by centrifugation, resuspended in saline solution, irradiated (1.2×10^5 J/m^2) and incubated post-illumination in plain TYB at 37°C. OD and nucleotide concentration were measured during growth delay as indicated in the text. ppGpp: (○) unirradiated and (●) irradiated. pppGpp: (△) unirradiated and (▲) irradiated cells.

by the radiation when cells were grown at 37°C (Table 2). But, when the SDH was measured during the growth delay of cells incubated at 30°C or grown pre-irradiation in the presence of either glycerol or sucrose, its specific activity proved considerably higher than the above (Table 3).

We found that the stringent response was triggered in all experimental conditions assayed, reporting here on intracellular accumulation of ppGpp and pppGpp and the relationship between their degradation and lag's extent. For that purpose cells were grown pre-irradiation in the presence of [^{32}P]orthophosphoric acid (see Materials and Methods) and the fate of labeled guanosine polyphosphates was followed in parallel with the growth delay induced by near-UV radiation. As depicted in Fig. 5, cells incubated in unsupplemented TYB accumulated ppGpp and pppGpp in a 2 : 1 ratio, with [^{32}P] counts in cpm/OD units increased fourfold at 0 time after the irradiation. It is also seen that both nucleotides followed similar degradation patterns during the growth delay measured in the absence of [^{32}P]. For that reason further references to pppGpp are omitted.

In table 4 we present the kinetics of [^{32}P]ppGpp degradation during the growth delay of cells incubated pre- and post-irradiation in plain TYB at 37°C, in comparison with: (i) cells grown pre-irradiation in TYB at 30°C and incubated post-irradiation at 43°C in the same medium, and (ii) cells grown pre-irradiation at 37°C in TYB supplemented with either 200 m*M* glycerol or 300 m*M* sucrose, with post-irradiation incubation at 43°C in unsupplemented TYB. Our results show a substantial ppGpp increase in unirradiated controls, i.e. log phase cells growing in rich medium with vigorous shaking, which were spun down, resuspended in cold 150 m*M* NaCl, and then kept 60 min in an ice bath. As seen in Table 4 the ppGpp excess found

Table 3. Effect of near-UV on SDH activity of *E. coli* K-12 grown in different pre-irradiation conditions

Pre-irrad. growth in E + succinate		SDH activity (nmol/mg/h)		
supplement	temp.	unirradiated	irradiated	% SDH inhibition
None	37°C	18.4	2.7	85
None	30°C	9.1	3.6	60
Sucrose	37°C	17.9	9.2	49
Glycerol	37°C	7.8	7.2	7

Cells were grown from an OD of 0.100 to about 0.800 in medium E + 7.5 m*M* sodium succinate, supplemented or not with 300 m*M* sucrose or 200 m*M* glycerol and incubated at either 37° or 30°C (see Table). Cells were irradiated (1.2×10^5 J/m^2) as described in Materials and Methods, resuspended at an OD of 0.600 in E + succinate (no additions), incubated 10 min at 37°C and the SDH activity measured in cell free extracts as indicated in the text.

Table 4. Effect of culture conditions on ppGpp degradation during the growth delay induced by near-UV in *E. coli* K-12

Growth delay min	Intracellular [^{32}P]-labeled ppGpp (cpm/μg cell. protein)							
	unsuppl. TYB 37°C		unsuppl. TYB 30°C		TYB + sucrose 37°C		TYB + glycerol 37°C	
	Unirr.	Irrad.	Unirr.	Irrad.	Unirr.	Irrad.	Unirr.	Irrad.
0	3.72	11.00	2.93	15.60	3.70	22.10	4.20	14.50
15	0.27	4.59	0.16	7.40	0.58	13.20	0.39	6.30
30	0.22	3.98	0.14	3.40	0.24	3.90	0.30	3.60
60	0.20	0.41	0.15	0.20	0.10	0.38	0.10	0.30
90	0.08	0.10	0.07	0.09	0.06	0.12	0.07	0.12

Cells were grown from an OD of 0.100 to 0.800 in the presence of [^{32}P]orthophosphoric acid in TYB supplemented or not with 300 m*M* sucrose or 200 m*M* glycerol at either 37° or 30°C (see above). Harvested by centrifugation, cells were irradiated (1.2×10^5 J/m^2) in saline solution as described in Materials and Methods, resuspended in TYB (no additions) and the growth delay followed at either 37°C (cells previously grown in plain TYB at 37°C) or at 43°C (all the other groups). ppGpp levels and cellular protein content were simultaneously measured as indicated in the text, the latter in precipitates saved after formic acid extraction.

at 0 time after illumination was quickly degraded, and cells recovered guanosine polyphosphate basal level shortly after reincubation.

The unexpected high concentration of guanosine polyphosphates found in unirradiated controls is attributed to the stress suffered by the cells with the drastic change in environment described above. In fact, the accumulation of ppGpp was originally observed in stringent but not in relaxed strains during amino acid deprivation (Cashel, 1969), and afterward it was shown that cells accumulate rather high levels of the nucleotide upon starvation of sources of carbon, potassium or phosphates, or upon "energy step down" (Lazzarini and Cashel, 1971; Goldberg and St. John 1976). Moreover, Cashel (1975) analyzed a relA independent ppGpp accumulation occurring in a variety of treatments like interruption of aereation (Lund and Kjeldgaard, 1972) and sodium chloride solutions (Harshman and Yamasaki, 1972), amongst others. Later, St. John and Goldberg (1978) reported accumulation of ppGpp when ATP production was lowered by electron transport inhibition. We suggest that the increased levels of ppGpp found in unirradiated cells were probably induced by one or more of the above factors.

Regarding irradiated cells, they showed at 0 time after UV-exposure an even higher nucleotide concentration than unexposed controls (Table 4) which was degraded with a half decay time close to 15 min. It is noteworthy that 60 min after near-UV exposure the intracellular ppGpp concentration was almost equivalent in all irradiated samples, regardless of temperature or medium composition. Yet, the growth delay extent showed a remarkable difference, since cells in unsupplemented TYB at 37°C suffered a 100 min lag while in all other conditions normal growth reinitiated about 40 min earlier, showing that only those whose envelope biochemical functions were restored to normality by the influence of environmental conditions were rescued from the growth lag.

In summary, we postulate that the growth delay induced by sublethal doses of 366 nm UV radiation, result from the association of membrane perturbation(s) with the photochemical cross-linking of tRNA's amplified by the stringent response. Changes in environmental conditions known to influence membrane properties affect biochemical functions determining modifications in the lag extent, allowing irradiated cells to resume normal growth. As seen on Table 4, 60 min was the post-irradiation time required for cells from all assayed conditions to return ppGpp concentration to basal levels, but only those recovering membrane normal activities were able to reinitiate normal growth, suggesting that 60 min could be the true lag required by cells in our experimental conditions to recover from the photochemical tRNA-stringent response effect.

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REFERENCES

Back, J., D. Oakenfull and M. Smith (1979) Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry* **18**, 5191–5196.

Blanchetot, A., E. Hajnsdorf and A. Favre (1984) Metabolism of tRNA in near-ultraviolet-illuminated *Escherichia coli*: The tRNA repair hypothesis. *Eur. J. Biochem.* **139**, 547–552.

Brodie, A. and J. Ballantine (1960) Oxidative phosphorylation in fractionated bacterial systems. *J. Biol. Chem.* **235**, 236–231.

Broekman, J. H. and J. F. Steenbakkers (1973) Growth in high osmotic medium of an unsaturated fatty acid auxotroph of *Escherichia coli* K-12. *J. Bacteriol.* **116**, 285–289.

Cashel, M. (1969) The control of ribonucleic acid synthesis in *Escherichia coli*. *J. Biol. Chem.* **224**, 3133–3141.

Cashel, M. (1975) Regulation of bacterial ppGpp and pppGpp. *Ann. Rev. Microbiol.* **29**, 301–318.

Favre, A. (1980) Croissance de la bactérie *E. coli* sous illumination a 366 nm. *C. R. Acad. Sci. Paris, Séries D,* **290**, 1111–1114.

Favre, A., F. Hajnsdorf, K. Thiam and A. Caldeira de Araujo (1985) Mutagenesis and growth delay induced in *Escherichia coli* by near-ultraviolet radiation. *Biochimie* **67**, 335–342.

Goldberg, A. L. and A. C. St. John (1976) Intracellular protein degradation in mammalian and bacterial cells: Part 2. *Ann. Rev. Biochem.* **45**, 747–803.

Hajnsdord, E. and A. Favre (1986) Metabolism of tRNAs in growing cells of *Escherichia coli* illuminated with near-ultraviolet light. *Photochem. Photobiol.* **43**, 157–164.

Harshman, R. B. and H. Yamasaki (1972) MS I accumulation induced by sodium chloride. *Biochemistry* **11**, 615–618.

Hederstedt, L. and L. Rutberg (1981) Succinate dehydrogenase. A comparative review. *Microbiol. Rev.* **45**, 542–555.

Ingram, L. O. (1982) Regulation of fatty acid composition in *Escherichia coli*: A proposed mechanism for changes induced by ethanol, chaotropic agents, and a reduction of growth temperature. *J. Bacteriol.* **149**, 166–172.

Jagger, J., W. Wise and R. S. Stafford (1964) Delay in growth and division induced by near ultraviolet radiation in *Escherichia coli* B and its role in photoprotection and liquid holding recovery. *Photochem. Photobiol.* **3**, 11–24.

Jagger, J. (1981) Near-UV radiation effects on microorganisms. *Photochem. Photobiol.* **34**, 761–768.

Kim, I. C. and P. D. Bragg (1971) Some properties of succinate dehydrogenase of *Escherichia coli*. *Can. J. Biochem.* **49**, 1098–1104.

Kubitschek, H. E. and R. J. Doyle (1981) Growth delay induced in *Escherichia coli* by near-ultraviolet radiation: relationship to membrane transport functions. *Photochem. Photobiol.* **33**, 695–702.

Lazzarini, R. A. and M. Cashel (1971) On the regulation of guanosine tetraphosphate levels in stringent and relaxed strains of *Escherichia coli*. *J. Biol. Chem.* **246**, 4381–4385.

Lowry, O. H., N. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–267.

Lund, E. and N. O. Kjeldgaard (1972) Metabolism of guanosine tetraphosphate in *Escherichia coli*. *Eur. J. Biochem.* **28**, 316–326.

Peak, M. J. and J. G. Peak (1980) Protection by glycerol against the biological actions of near-ultraviolet light. *Rad. Res.* **83**, 553–558.

Peak, M. J. and J. G. Peak (1982) Single strands breaks induced in *Bacillus subtilis* DNA by ultraviolet light: Action spectrum and properties. *Photochem. Photobiol.* **35**, 675–680.

Peak, J. G., M. J. Peak and C. S. Foote (1982) Effects of glycerol upon the biological actions of near-ultraviolet light: spectra and concentration dependence for transforming DNA and for *Escherichia coli* B/r. *Photochem. Photobiol.* **36**, 413–416.

Pizarro, R. A., G. O. Boselli and L. V. Orce (1985) Biochemical and phenotypical correction of an envelope mutant of *Salmonella typhimurium*. *Arch. Int. Physiol. Biochem.* **93**, 333–337.

Quinn, P.J. (1981) The fluidity of cell membranes and its regulation. *Prog. Biophys. Molec. Biol.* **38**, 1–104.

Ramabhadran, T. V. and J. Jagger (1976) Mechanism of growth delay induced in *Escherichia coli* by near-ultraviolet radiation. *Proc. Natl. Acad. Sci. USA* **73**, 59–69.

Ramabhadran, T. V., P. Fossum and J. Jagger (1976) *Escherichia coli* mutant lacking 4-thiouridine in its transfer ribonucleic acid. *J. Bacteriol.* **128**, 671–672.

Sharma, R. and J. Jagger (1981) Ultraviolet (254–405) action spectrum and kinetic studies of alanine uptake in *Escherichia coli* B/r. *Photochem. Photobiol.* **33**, 173–177.

Sprott, G., W. Martin and H. Schneider (1976) Differential effects on near-UV and visible light on active transport and other membrane processes in *Escherichia coli*. *Photochem. Photobiol.* **24**, 21–27.

St. John, A. C. and A. L. Goldberg (1978) Effects of reduced energy production on protein degradation, guanosine tetraphosphate and RNA synthesis in *Escherichia coli*. *J. Biol. Chem.* **246**, 4381–4385.

Taber, H., B. J. Pomerantz and G. M. Halfenger (1978) Near ultraviolet induction of growth delay studied in a menaquinone-deficient mutant of *Bacillus subtilis*. *Photochem. Photobiol.* **28**, 191–196.

Thiam, K. and A. Favre (1984) Role of the stringent response in the expression and mechanism of near-ultraviolet induced growth delay. *Eur. J. Biochem.* **145**, 137–142.

Thomas, G. and A. Favre (1975) 4-Thiouridine as a target for near-ultraviolet light induced growth delay in *Escherichia coli*. *Biochem. Biophys. Res. Comm.* **66**, 1454–1460.

Thomas, G. and A. Favre (1980) 4-Thiouridine triggers both growth delay induced near-UV light and photoprotection. *Eur. J. Biochem.* **113**, 67–74.

Thompson, G. A. (1980) *The Regulation of Membrane Lipid Metabolism*. CRC Press, Boca Raton, FL.

Vogel, H. J. and D. M. Bonner (1956) Acetyl-ornithinase of *Escherichia coli*: partial purification and some properties. *J. Biol. Chem.* **218**, 97–106.