

Gene expression of *Escherichia coli* in continuous culture during adaptation to artificial sunlight

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Summary

***Escherichia coli* growing in continuous culture under continuous UVA irradiation exhibits growth inhibition with a subsequent adaptation to the stress. Transcriptome analysis was performed during transient growth inhibition and in the UVA light-adapted growth state. The results indicate that UVA light induces stringent response and an additional response that includes the upregulation of the synthesis of valine, isoleucine, leucine, phenylalanine, histidine and glutamate. The induction of several SOS response-genes strongly points to DNA damage as a result of UVA exposure. The involvement of oxidative stress was observed with the induction of *ahpCF*. Taken together it supports the hypothesis of the production of reactive oxygen species by UVA light. In the UVA-adapted cell population strong repression of the acid tolerance response was found. We identified the enzyme chorismate mutase as a possible chromophore for UVA light-inactivation and found strong repression of the *pyrBI* operon and the gene *mgfA* encoding for an ATP-dependent Mg²⁺ transporter. Furthermore, our results indicate that the role of RpoS may not be as important in the adaptation of *E. coli* to UVA light as it was implicated by previous results with starved cells, but that RpoS might be of crucial importance for the resistance under transient light exposure.**

Introduction

Sunlight has long been recognized as a harmful radiation that is capable of damaging microorganisms (Downes, 1886). In the environment, during transmission between hosts, enteric bacteria are often exposed to sunlight (Calkins and Thordardottir, 1980). The deleterious effect

of sunlight on enteric bacteria has been used to develop simple drinking water disinfection methods like solar disinfection (SODIS) (Acra *et al.*, 1984; Wegelin *et al.*, 1994). Today, SODIS is one of the recommended methods for household drinking water disinfection (WHO/UNICEF, 2005). However, the exact mechanism(s) of disinfection are not yet known. The two primary factors for bacterial inactivation in this method are believed to be mild heat and UVA light.

The spectrum of solar UV light is divided into three wavelength ranges called UVC (200–290 nm) or far-UV (FUV), UVB 290–320 nm or mid-UV, and UVA (320–400 nm) or near-UV (NUV) (Jagger, 1985). In other publications UVB and UVA is collectively referred to as NUV (290–400 nm) (Eisenstark, 1989). On the following pages NUV and UVA are used as synonyms for the wavelength range between 320 and 400 nm (according to Jagger). The response of enteric bacteria, particularly *Escherichia coli*, to irradiation of solar UV light has been investigated for more than 70 years. A first physiological study on the lethal action (no recovery on standard agar) of NUV light on starved bacterial cultures was conducted by Hollaender (1943) followed by studies on the growth kinetics of *E. coli* under sublethal (recovery on standard or non-standard agar) NUV irradiation intensities (Jagger *et al.*, 1964; Phillips *et al.*, 1967). A well examined type of sublethal effects in *E. coli* is the phenomenon of growth delay (Jagger *et al.*, 1964). It was shown that growth delay is largely due to absorption ($\lambda_{\max} = 340$ nm) by 4-thiouridine (s⁴U), an unusual base occurring in the 8-position in 65% of tRNA species of *E. coli* (Favre *et al.*, 1985; Jagger, 1985). The relative importance of DNA and membrane damage in the lethal and sublethal actions of NUV light on *E. coli* are still unclear. In the early seventies it was demonstrated that NUV inactivation is oxygen-dependent (Eisenstark, 1970; Webb and Lorenz, 1970). This finding was followed by many studies showing that NUV light produces reactive oxygen species (ROS) via sensitization of endogenous photosensitizers (e.g. flavin and haeme groups) and that intracellular iron pools may be involved as well (McCormick *et al.*, 1976; Hartman and Eisenstark, 1978; Webb and Brown, 1979; Ahmad, 1981; Eisenstark, 1998). Reactive oxygen species can harm proteins as well as nucleic acids and therefore the lethal actions of NUV light are complex. It was found that broad-spectrum NUV light can block the electron transport chain, inactivates

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transport systems, interferes with metabolic energy production and can cause a general increase in permeability of the membrane (Koch *et al.*, 1976; Jagger, 1981; Berney *et al.*, 2006a). Numerous proteins, e.g. catalases (HPI and HPII), dihydroxyacid dehydratase (DHAD) and ribonucleotide reductase were identified to be NUV-sensitive (Eisenstark, 1989; 1998). The same author listed more than 20 different genes, which might be involved in protection against NUV light. These studies were conducted with either strains carrying a mutation or a reporter gene fusion at the specific gene location. In a recent study, Qui and colleagues (Qiu *et al.*, 2005) found that *Shewanella oneidensis* expressed twice as many genes after UVA light exposure than after UVC treatment. This result points to a high complexity of UVA light-induced stress response.

Although many pieces of the puzzle seem to be available, a comprehensive understanding of all processes involved in the response of *E. coli* to UVA light is still lacking. Recent advances in molecular microbiology allow analysis of differential gene expression of the whole genome in a number of microorganisms. Therefore, we used the microarray technology, to screen global gene expression in *E. coli* in response to UVA light. Recently we have shown that *E. coli* growing in continuous culture initially showed growth inhibition but then adapted to UVA irradiation with a fluence-rate of 50 W m^{-2} (Berney, 2006). In order to investigate the process of initial growth inhibition and subsequent adaptation of these cells, we analysed the gene expression pattern in an unadapted (after 1 h of irradiation) and in an adapted population of *E. coli* (after 50 h of irradiation).

Results and discussion

Escherichia coli K12 MG1655 was grown in continuous culture in two bioreactors at a dilution rate of 0.5 h^{-1} and was directly irradiated with UVA light (50 W m^{-2}) (Fig. 1). Two aluminum foil-wrapped control bioreactors were run with the same feed medium at the identical dilution rate.

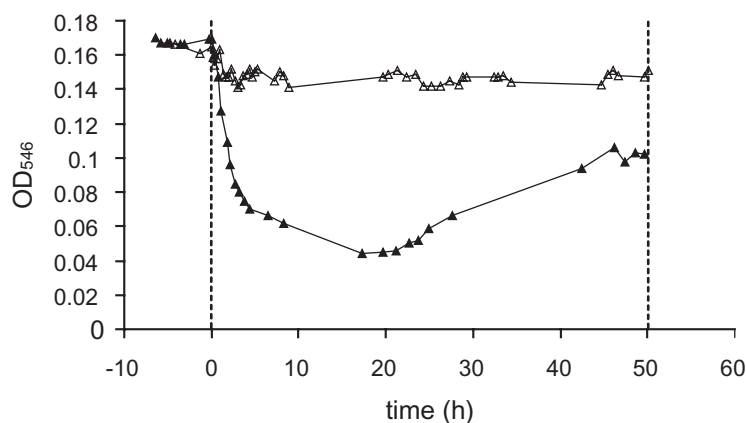


Fig. 1. *Escherichia coli* K12 MG1655 growing at a dilution rate of 0.5 h^{-1} in continuous culture was exposed to UVA light at 50 W m^{-2} and optical density was measured throughout the experiment (▲). A control bioreactor (aluminum foil-wrapped) was run at 0.5 h^{-1} (△) with the same feed medium. Samples for gene array analysis were taken after 1 h and 50 h. Here only the data from the irradiated continuous culture running for 50 h are shown.

Immediately after starting irradiation optical density in the bioreactor decreased significantly indicating that bacteria stopped growing and were washed out (Fig. 1). Between 10 and 20 h of irradiation optical density in the culture stabilized and then started to increase again and after approximately 50 h specific growth rate was back on the initial level (0.5 h^{-1}) indicated by the new steady state that was reached. Optical density of the continuous culture in the new steady state was significantly lower than before irradiation. A thorough discussion of the physiological changes during this adaptive response was presented recently (Berney, 2006).

Global gene expression in *E. coli* during adaptation to UVA irradiation

The global transcriptional profile of the *E. coli* population harvested from the continuous cultures that were irradiated with UVA light for either 1 h or 50 h was analysed with the microarray technology. After 1 h a total of 312 genes were differentially expressed in the irradiated cells compared with the non-irradiated control population. Upregulated were 163 genes [induction factor (IF) ≥ 2 , *t*-test *P*-value ≤ 0.05] and 149 genes were downregulated (IF ≤ -2). After 50 h a total of 193 differentially expressed genes (100 upregulated, 93 downregulated) were detected, and 52 of these genes were differentially expressed at both time points (Appendix S1). All differentially expressed genes detected after 1 h or 50 h of irradiation were distributed into nine functional groups according to the EcoCyc annotation (<http://ecocyc.org/>) (Fig. 2). The tables shown in this paper list selected genes collected from different functional groups.

Amino acid biosynthesis

One of the most striking results of this analysis was perhaps that after 1 h of irradiation 37 genes involved in amino acid metabolism, mostly amino acid biosynthesis,

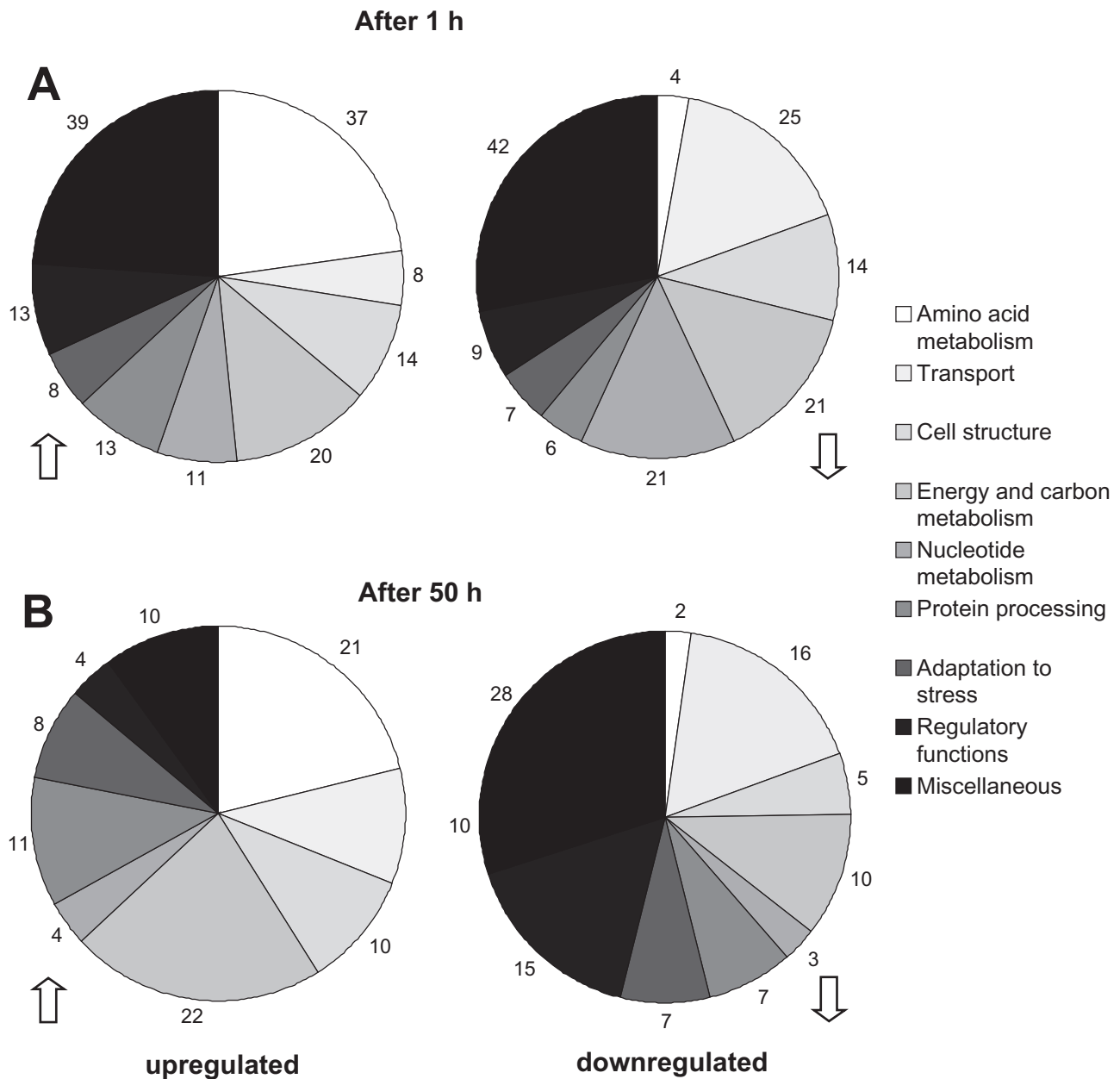


Fig. 2. Classification of genes differentially expressed in *E. coli* MG1655 grown in 'LB-limited' continuous culture at a dilution rate of $D = 0.5 \text{ h}^{-1}$ and irradiated with UVA light (50 W m^{-2}) for (A) 1 h or (B) 50 h. Only genes that were upregulated by a factor ≥ 2 or downregulated by a factor ≤ -2 are displayed. Only genes with P -values ≤ 0.05 were selected.

were upregulated while only four genes in this category were downregulated (Fig. 2). The same pattern was also observed after 50 h with 21 up- and two downregulated genes. Among these, genes involved in biosynthesis of histidine, valine, isoleucine, leucine, glutamate and phenylalanine were upregulated in both physiological states while the superpathway of cysteine biosynthesis and sulfate assimilation was not induced after 50 h (Table 1). The increased mRNA levels for amino acid biosynthesis in growth arrested (after 1 h irradiation) as well as in the

adapted cells (after 50 h) indicate that either the uptake or/and the biosynthesis of these amino acids is hampered. However, the situation is confusing. On one hand, the inhibition of both the leucine and the leucine-isoleucine-valine (LIV) systems in *E. coli* K-12 by UVA light was shown by Robb and colleagues (Robb *et al.*, 1978). The same authors provided a UV action spectrum for the two uptake systems showing a distinct peak at 365 nm. On the other hand, it was found that the addition of branched-chain amino acids (leucine, isoleucine and valine) to the

Table 1. *Escherichia coli* genes involved in amino acid synthesis whose expression levels were increased by a factor ≥ 2 or decreased by a factor ≤ -2 in cultures exposed for either 1 or 50 h to UVA light in 'LB-limited' continuous culture at $D = 0.5 \text{ h}^{-1}$.

Gene no.	Name	Gene product	Induction factor	
			1 h	50 h
Histidine biosynthesis				
B2024	<i>hisA</i>	n-(5'-phospho-L-ribosyl-formimino)-5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide isomerase	5.1	3.9
B2022	<i>hisB</i>	Imidazoleglycerolphosphate dehydratase and histidinol-phosphate phosphatase	3.4	2.8
B2021	<i>hisC</i>	Histidinol-phosphate aminotransferase	4.0	2.4
B2020	<i>hisD</i>	L-Histidinal:nad ⁺ oxidoreductase, L-histidinol:nad ⁺ oxidoreductase	5.6	3.0
B2025	<i>hisF</i>	Imidazole glycerol phosphate synthase subunit in heterodimer with hisH = imidazole glycerol phosphate synthase holoenzyme	4.0	2.9
B2019	<i>hisG</i>	ATP phosphoribosyltransferase	3.8	2.4
B2023	<i>hisH</i>	Glutamine amidotransferase subunit of heterodimer with hisF = imidazole glycerol phosphate synthase holoenzyme	2.5	
B2026	<i>hisI</i>	Phosphoribosyl-amp cyclohydrolase, phosphoribosyl-ATP pyrophosphatase	3.9	2.9
B2018	<i>hisL</i>	His operon leader peptide	2.2	
Valine, isoleucine, leucine biosynthesis				
B0071	<i>leuD</i>	Isopropylmalate isomerase subunit	2.5	
B0072	<i>leuC</i>	3-Isopropylmalate isomerase (dehydratase) subunit		2.4
B0073	<i>leuB</i>	3-Isopropylmalate dehydrogenase		6.0
B3670	<i>ilvN</i>	Acetolactate synthase i, valine-sensitive, small subunit	7.7	6.7
B3671	<i>ilvB</i>	Acetolactate synthase i, valine-sensitive, large subunit	18.1	6.0
B3672	<i>ilvL</i>	Ilvb operon leader peptide	5.6	2.6
B3766	<i>ilvL</i>	Ilvgeda operon leader peptide	2.4	
B3767	<i>ilvG</i>	Acetolactate synthase ii, large subunit	10.5	2.5
B3769	<i>ilvM</i>	Acetolactate synthase ii, valine-insensitive, small subunit	4.7	
B3770	<i>ilvE</i>	Branched-chain amino acid aminotransferase		2.0
Superpathway of sulfate assimilation and cysteine biosynthesis				
B2752	<i>cysD</i>	ATP : sulfurylase (ATP : sulfate adenyltransferase), subunit 2	6.8	
B2763	<i>cysI</i>	Sulfite reductase, alpha subunit	6.9	
B2764	<i>cysJ</i>	Sulfite reductase (NADPH), flavoprotein beta subunit	3.3	
B2421	<i>cysM</i>	Cysteine synthase b, <i>o</i> -acetylserine sulfhydrylase b	2.1	
B2751	<i>cysN</i>	ATP-sulfurylase (ATP : sulfate adenyltransferase), subunit 1, probably a GTPase	2.3	
B2425	<i>cysP</i>	Thiosulfate binding protein	2.3	
B2423	<i>cysW</i>	ABC-type sulfate transport system, permease component	3.9	
Glutamate biosynthesis				
B3212	<i>gltB</i>	Glutamate synthase, large subunit	5.2	4.7
B3213	<i>gltD</i>	Glutamate synthase, small subunit	2.1	2.3
B3214	<i>gltF</i>	Regulator of <i>gltd</i> operon, induction of <i>ntr</i> enzymes		-22.0
Phenylalanine biosynthesis				
B2599	<i>pheA</i>	Chorismate mutase-p and prephenate dehydratase	9.4	19.2

Genes were only listed if the *P*-value of the three replicates was ≤ 0.05 .

medium when exposing *thil*-minus mutants of *Salmonella typhimurium* to NUV greatly delayed the entry into the growth lag, whereas other amino acids (methionine, arginine and glutamate) had no effect (Kramer *et al.*, 1988). These authors proposed that the NUV-induced growth delay in this strain may involve damage to branched-chain amino acid biosynthesis. Furthermore, the initial target of hyperbaric O₂ toxicity has been shown to be LIV biosynthesis, i.e. the addition of these amino acids protected *E. coli* from this stress (Boehm *et al.*, 1976; Brown and Seither, 1983). This indicates that although amino acid uptake systems are compromised by UVA light, this seems not to affect all systems and bacterial cells can still take up a certain amount of amino acids. Luria-Bertani (LB) medium that was used in our experiments contains yeast extract, which itself was shown to contain enough amino acids to protect *E. coli* E26 from oxygen toxicity

(Boehm *et al.*, 1976). Hence, our results probably indicate both, an inhibition of amino acid biosynthesis and an inhibition of amino acid uptake systems with subsequent overexpression of the biosynthesis genes. After 1 h of irradiation the gene *brnQ* was downregulated by a factor of -2.2. It codes for a branched-chain amino acid transport system ii carrier protein, corresponding to the Liv-II branched-chain amino acid transport system in *E. coli*, which has been shown to transport leucine, valine and isoleucine. On the other hand, *livK*, which codes for the periplasmic binding protein of the high-affinity leucine-specific transport system, was upregulated by a factor of 3.1. In the adapted population though (after 50 h), these systems were not differentially expressed indicating that this response is only transient and perhaps secondary.

The *pheA* gene encodes two enzymes, chorismate mutase and prephenate dehydratase, both involved in

phenylalanine biosynthesis. It was found that prephenate bound in the active site of chorismate mutase absorbs light around 340 nm (Roitberg *et al.*, 2000). This might inactivate the enzyme and explains the strong upregulation during adaptation ($IF_{1h} = 9.4$; $IF_{50h} = 19.2$). Furthermore, histidine was shown to be sensitized in UVA-exposed *S. typhimurium* (Rahman *et al.*, 1995). These authors suggest that histidine first reacts with singlet oxygen to produce an endoperoxide, which decomposes to a complex mixture of products, some of which may be genotoxic. The expression of the *his* operon of *S. typhimurium* is governed by at least two independent mechanisms: (i) an operon-specific 'activator-attenuator' mechanism and (ii) a mechanism involving ppGpp as a positive effector (Venetianer, 1969; Stephens *et al.*, 1975). The same authors showed that an intracellular upshift of ppGpp concentration resulted in the induction of the *his* operon and proposed that ppGpp is a component of a sensing mechanism for adjusting the synthesis of histidine biosynthetic enzymes linking the need for histidine with the availability of all of the amino acids from the external environment. To date, the suggestion that this mechanism is similar for other amino acids has not been proven. In fact, the contrary seems more likely because Chang and colleagues (Chang *et al.*, 2002) measured a general downregulation of amino acid biosynthesis genes in *E. coli* during growth arrest after H₂O₂ addition in batch culture. In this respect our results indicate that the upregulation of biosynthesis of isoleucine, leucine, valine, phenylalanine and glutamate is indeed important in the UVA light-triggered stress response and is not only a result of the stringent response. Also the role of histidine could be important in the UVA stress response because the *his* operon was still overexpressed in the adapted cell population (Table 1). So here, in contrast to 'growth arrest' experiments we see an upregulation of amino acid biosynthesis genes despite the fact that the cells significantly slowed down growth. Hence, this is a strong indication that it is a distinctively different stress response.

The NADH-dependent glutamate synthase (encoded by *gltB*: $IF_{1h} = 5.2$, $IF_{50h} = 4.2$ and *gltD*: $IF_{1h} = 2.1$, $IF_{50h} = 2.3$) converts L-glutamine and alpha-ketoglutarate into two L-glutamate molecules (Castano *et al.*, 1988). L-Glutamate is an essential amino acid in the assimilation of ammonia and for the biosynthesis of other amino acids (e.g. phenylalanine), for NAD biosynthesis or purine and pyrimidine *de novo* biosynthesis. Surprisingly, GltF, which is thought to be a regulator for the *gltBDF* operon, was downregulated 22-fold but the genes *gltB* and *gltD* were upregulated. However, it was shown that the expression of *gltB* and *gltD* is not directly regulated by GltF and that the function of GltF is still elusive (Goss *et al.*, 2001). Our results rather suggest that GltF might be involved in the acid stress response. The induction of glutamate synthase

after 1 h as well as after 50 h of irradiation (Table 1) indicates that glutamate is an important amino acid in UVA stress resistance and, therefore, glutamate decarboxylase (encoded by *gadA*, $IF_{50} = -14.9$) might be repressed. Glutamate synthase together with glutamine synthetase (encoded by *glnA*, $IF_1 = -3.2$) is part of the high-affinity ammonia assimilation pathway (GS-GOGAT) in *E. coli* (Reitzer, 2003). Its upregulation indicates cell internal N-limitation. Hence, inhibition of nitrogen-assimilation might be another reason for the upregulation of amino acid biosynthesis because nitrogen is an essential molecule in amino acids.

Adaptation to stress

Eisenstark (1989) proposed to divide the complex mechanism in *E. coli* for coping with UVA light into two parts: (i) detoxification of reactive molecules that result from photooxidation (reactive oxygen species); (ii) repair of DNA damage and re-synthesis of damaged tRNA. We suspect that this is not the whole story and that mechanisms like (iii) the repair or the reaction to the inhibition of components like transport of amino acids (and perhaps others) is an additional aspect.

The DNA damage caused by UVC radiation is known to induce a cellular protective response known as SOS response. The *recA* gene plays a central role in the regulation of the SOS response. Whether UVA radiation can also induce a similar SOS response in bacterial cells has been a subject of controversy. Neither monochromatic light (335 nm or 365 nm) nor broad-spectrum solar UV resulted in derepression of the *recA* promoter and subsequent SOS function in *E. coli* (Turner and Eisenstark, 1984). These authors proposed that the specific NUV-induced DNA lesions, and particularly single-strand DNA breaks, are unable to trigger the SOS response because they bind the RecA protein but fail to activate it. Others reported that the inducibility of the SOS response under NUV stress was 10–20 times higher in the *thil* mutant than in the parent strain (*thil*⁺) of *E. coli* AB1157 (Caldeira de Araujo and Favre, 1986). These same authors suggested that the growth delay effect resulting from exposure to UVA light was actually responsible for reducing the SOS response. Similar results were obtained with *S. typhimurium* TA 1535 containing multiple copies of plasmid psK1002 carrying a *umuC-lacZ* fusion gene (Rahman *et al.*, 1995). Our results show a clear induction of the *recA* gene in both populations ($IF_{1h} = 3.1$, $IF_{50h} = 6.7$) (Table 2). Accordingly, *recN* ($IF_{1h} = 5.7$, $IF_{50h} = 6.8$) was upregulated at both time points. The induction of *dinD* ($IF_{50h} = 5.4$) and *dinI* ($IF_{50h} = 3.2$) genes in the adapted culture is another indication that the SOS response is involved in the UVA stress response. Both genes encode for DNA damage-inducible proteins and are

Table 2. Selected *E. coli* genes involved in stress response, whose expression levels were increased by a factor ≥ 2 or decreased by a factor ≤ -2 in cultures exposed for either 1 or 50 h to UVA light in 'LB-limited' continuous culture at $D = 0.5 \text{ h}^{-1}$.

Gene no.	Name	Product and function	Induction factor	
			1 h	50 h
DNA damage inducible				
B3645	<i>dinD</i>	DNA-damage-inducible protein		5.4
B1061	<i>dinI</i>	Damage-inducible protein i		3.2
B2699	<i>recA</i>	DNA strand exchange and renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease	3.1	6.7
B2616	<i>recN</i>	Protein used in recombination and DNA repair	5.7	6.8
B1863	<i>ruvC</i>	Holliday junction nuclease, resolution of structures, repair	2.2	
B2009	<i>sbmC</i>	sbmC protein. Gene expression is increased at stationary phase and by treatment with compounds that cause DNA damage	2.3	
B0958	<i>sulA</i>	Suppressor of lon, inhibits cell division and ftsZ ring formation	3.8	5.6
B1183	<i>umuD</i>	SOS mutagenesis, error-prone repair, processed to umud', forms complex with umuc	3.5	
B1848	<i>yebG</i>	Hypothetical protein; gene is part of SOS regulon (YebG)		3.5
Cold/Heat shock				
B3556	<i>cspA</i>	Cold-shock protein 7.4, transcriptional activator of hns	-2.6	
B1558	<i>cspF</i>	Cold-shock protein	-3.1	
B0990	<i>cspG</i>	Homologue of <i>Salmonella</i> cold-shock protein	-3.4	
B0015	<i>dnaJ</i>	Chaperone with dnaK, heat-shock protein		2.7
B1967	<i>hchA</i>	Heat-shock protein (Hsp) 31 (HchA)		-2.1
B3687	<i>ibpA</i>	Heat-shock protein	-2.2	
B3686	<i>ibpB</i>	Heat-shock protein	-2.6	-2.2
Oxidative stress				
B0605	<i>ahpC</i>	Alkyl hydroperoxide reductase, c22 subunit, detoxification of hydroperoxides		2.2
B0606	<i>ahpF</i>	Alkyl hydroperoxide reductase, f52a subunit, detoxification of hydroperoxides	2.3	4.2
B1732	<i>katE</i>	Catalase, hydroperoxidase hpII(iii)		-2.3
B3908	<i>sodA</i>	Superoxide dismutase, manganese	-2.7	
Stringent response				
B0029	<i>ispH</i>	Control of stringent response, involved in penicillin tolerance	2.2	
B3779	<i>gppA</i>	Guanosine pentaphosphatase, exopolyphosphatase, requires Mg ²⁺	-2.4	
UV response				
B1895	<i>uspC</i>	Universal stress protein with a role in resistance to UV irradiation	2.2	
General stress response				
B2741	<i>rpoS</i>	RNA polymerase, sigma s (sigma38) factor, synthesis of many growth phase-related proteins	2.8	

Genes were only listed if the *P*-value of the three replicates was ≤ 0.05 .

part of the SOS response in *E. coli* (Quillardet *et al.*, 2003). Another expressed gene product of the SOS response in *E. coli* is SulA ($IF_{1h} = 3.8$, $IF_{50h} = 5.6$), a cell division inhibitor that inhibits septation by interacting with FtsZ, a component of the cell division apparatus (Huisman and D'Ari, 1981; Jones and Holland, 1985). In wild-type cells, SulA is very unstable. This normal instability of SulA permits the cell to resume cell division once the environmental stress has been alleviated (Trempy and Gottesman, 1989). Previously we observed a cell size increase in UVA light-stressed *E. coli* cells, which was measured with flow cytometry (Berney, 2006). Even the UVA light-adapted cells showed increased cell size although they had resumed cell division. This indicates that SulA does not entirely inhibit cell division under continuous UVA light exposure, but it may transiently do so or lead to bigger cells when its concentration is higher.

There are some indications in our data supporting the hypothesis that the UVA inactivation mechanism includes oxidative stress. The *ahpCF* operon was upregulated especially in the adapted cells after 50 h of irradiation

($IF_{ahpC} = 2.2$, $IF_{ahpF} = 4.2$). This operon encodes alkylhydroperoxidase reductase (Ahp) in *E. coli* as well as in *S. typhimurium* (Jacobson *et al.*, 1989). The enzyme converts lipid hydroperoxides and other alkyl hydroperoxides to the corresponding alcohols, using either NADH or NADPH as the reducing agent. It was shown that Ahp is induced by oxidative stress in *E. coli* and *S. typhimurium* (Storz *et al.*, 1989). Interestingly, hydroperoxidase II (KatE) was downregulated in adapted cells ($IF_{50h} = -2.2$). Catalase is normally expressed under oxidative stress with H₂O₂ (Loewen and Triggs, 1984) but was shown to be directly inactivated by UVA light (Zigman *et al.*, 1996). This is supported by the fact that *E. coli* mutants carrying an insertion in the *katE* or *katG* locus were not more sensitive to UVA light than the parent strain (Sammartano *et al.*, 1986). The expression of the *katE* gene is regulated by RpoS (Visick and Clarke, 1997). RpoS, or σ^s , is a sigma subunit of RNA polymerase in *E. coli* that is induced in batch culture during transition from fast to slow growth (Hengge-Aronis, 2002) or in continuous culture as a function of specific growth rate (Ihssen and Egli, 2004).

Table 3. *Escherichia coli* genes involved in acid stress response, whose expression levels were increased by a factor ≥ 2 or decreased by a factor ≤ -2 in cultures exposed for either 1 or 50 h to UVA light in 'LB-limited' continuous culture at $D = 0.5 \text{ h}^{-1}$.

Gene no.	Name	Product and function	Induction factor	
			1 h	50 h
B3517	<i>gadA</i>	Glutamate decarboxylase isozyme		-14.9
B1492	<i>gadC</i>	Acid sensitivity protein, putative transporter		-14.8
B3512	<i>gadE</i>	GadE transcriptional activator, GadE overproduction causes resistance to low pH		-13.2
B3515	<i>gadW</i>	GadW transcriptional repressor; involved in acid resistance		-11.5
B3516	<i>gadX</i>	GadX transcriptional activator (YhiX)		-6.6
B3510	<i>hdeA</i>	Acid-resistance periplasmic protein, possible chaperone (HdeA)		-8.0
B3509	<i>hdeB</i>	10K-L protein, related to acid resistance protein of <i>Shigella flexneri</i>		-6.5
B3511	<i>hdeD</i>	Protein involved in acid resistance (HdeD)		-7.9
B3514	<i>mdtF</i>	Component of YhiUV multidrug transporter	-2.1	
B3506	<i>slp</i>	Outer membrane protein induced after carbon starvation, involved in the resistance to low pH		-5.8
B3508	<i>yhiD</i>	Putative transport ATPase		-2.4
B3504	<i>yhiS</i>	orf, hypothetical protein		-6.7
B3513	<i>yhiU</i>	Subunit of YhiUV multidrug transporter		-6.0

Genes were only listed if the *P*-value of the three replicates was ≤ 0.05 .

More than 400 genes are under RpoS control, many of them involved in responses to various environmental stresses (Weber *et al.*, 2005). In our experiment the *rpoS* gene was upregulated during growth inhibition at the start of irradiation (IF = 2.8). This was expected as intracellular RpoS concentration is dependent on specific growth rate and increases as specific growth rate decreases (Notley and Ferenci, 1996; Ihssen and Egli, 2004). The repression of *katE* in the UVA-adapted cell population might indicate a repression of *rpoS*. However, the regulation of RpoS levels in *E. coli* is very complex because it is also regulated at the translational level (Hengge-Aronis, 2002). When comparing the list of differentially expressed genes in our experiments (Appendix S1) with the core group of 140 genes, which were reported to be σ^S -controlled under three different growth and stress conditions (Weber *et al.*, 2005), only 13 of these genes were upregulated and one was downregulated after 1 h of UVA irradiation. In the adapted cell population (50 h) even 15 σ^S -controlled genes were downregulated. The control with real time polymerase chain reaction (RT-PCR) showed an IF of -1.8 for the *rpoS* gene compared with -1.1 in the microarray. This indicates that the global stress regulator RpoS is repressed rather than induced in UVA light-adapted cells. Starved cultures of an *E. coli rpoS*-minus strain were more sensitive to UVA, NUV and sunlight (Sammartano *et al.*, 1986; Berney *et al.*, 2006b). Starved or slow growing cells, though, have a very low protein turnover and, therefore, might not be able to rapidly adjust to UVA stress (Trempey and Gottesman, 1989; Berney, 2006). Hence, the general stress response initiated by RpoS before the entrance of the cells into stationary phase makes them more resistant to UVA light than the *rpoS*-minus cells by an unknown mechanism. However, in a culture growing under UVA light-stress the role

of RpoS might be different from that in stationary-phase cells.

Among the downregulated genes under RpoS control in the adapted cell population, we found 12 genes that are involved in acid stress resistance (Table 3). These genes are crucial for acid resistance and include *gadA*, *gadC* and the *hde* genes (as well as other less-well-characterized genes), together with their regulatory genes *gadE*, *gadX* and *gadW*. The regulatory network of acid tolerance is large, which might reflect the importance of acid stress resistance in the life cycle of *E. coli* (Hommais *et al.*, 2004). Table 3 is almost identical with the list of σ^S -controlled acid resistance genes published by Weber and colleagues (Weber *et al.*, 2005). Surprisingly, these genes were clearly stationary-phase-induced in a σ^S -dependent manner but exhibited only a minor or no σ^S -dependence upon acidic shift (Weber *et al.*, 2005). In our study these acid resistance genes showed no σ^S -dependent upshift after 1 h although the *rpoS* gene was upregulated (IF = 2.8). On the other hand, the same genes were strongly downregulated in the UVA-adapted cell population after 50 h of irradiation although *rpoS* expression was not affected. The pH in the UVA light-irradiated bioreactors was 7.2 ± 0.1 after 50 h while in the non-irradiated control bioreactor we measured $\text{pH} = 7.6 \pm 0.1$. This are clearly not acidic conditions. Hommais and colleagues (Hommais *et al.*, 2001) reported strong derepression of acid resistance genes in *hns* mutants. Nevertheless, in our experiments the IF of the *hns* gene was IF = 1 in both cases. One could speculate that the strong repression of acid tolerance genes seen in our experiment is a result from an upregulation in the control culture. However, no induction of acid tolerance genes was observed in cells from a glucose-limited culture having been cultivated under such conditions for 40 h (A. Franchini and T. Egli,

submitted). The GadE regulator was reported to be essential for the expression of *gadA*, *gadBC* and the *hde* genes (Hommais *et al.*, 2004) and, therefore, the high repression of the *gadE* gene is consistent with the observed repression of other acid stress-related genes.

Nucleotide metabolism and tRNA synthesis

In the experiments presented here we investigated the global regulatory profile of two different physiological states of *E. coli* growing in continuous culture under UVA irradiation. The first physiological state (after 1 h of irradiation) shows strong similarities with the state of growth inhibition during batch culture exposure to NUV light. It has been shown that growth inhibition is largely due to absorption by 4-thiouridine (s^4U) (Jagger, 1985) with the resultant production of a cross-link (Favre *et al.*, 1985). These cross-linked tRNAs have been shown to be poor substrates for amino acid-charging and, therefore, the amino acid availability is lowered and causes a shut-off of net RNA synthesis, resulting in the so-called stringent response (Ramabhadran and Jagger, 1976; Favre and Hajnsdorf, 1983). The stringent response serves as a control mechanism that reduces the cellular protein synthesis capacity when substrates for protein synthesis get scarce (Wick and Egli, 2004). The stringent response is mediated by the alarmones pppGpp and ppGpp, the intracellular levels of which are regulated by the enzymes RelA, SpoT, Gpp and Ndk. All these genes were not significantly upregulated in our experiments. The primary characteristic of the stringent response is the decrease in stable RNA levels. Further effects of (p)ppGpp accumulation are the induction of RpoS, inhibition of active transport of several metabolites and especially the enhanced transcription of some amino acid biosynthesis enzymes (Wick and Egli, 2004). Several of these effects were also

observed in our experiment whereas others are lacking. Transcription of the *rpoS* gene was induced (only after 1 h), biosynthesis of several amino acids (see above) was enhanced and transport systems like the phosphate ABC transporter (encoded by the *pstABCS* operon) were repressed (Appendix S1). In fact, transcription of 25 genes that are associated with transport processes was downregulated compared with only eight upregulated genes in this category. Also the *de novo* biosynthesis of purine, pyridine and pyrimidine ribonucleotides was strongly repressed (Table 4). Particularly, strong repression was observed for the genes of the pyrimidine ribonucleotide biosynthesis pathway *carA*, *carB*, *pyrB*, *pyrC*, *pyrD*, *pyrH*, *pyrI* and *pyrL* (Table 4) whereas the repression of purine biosynthesis was not as apparent. An almost identical result was found by Chang and colleagues (2002) in their growth arrest experiment with *E. coli* triggered by the addition of H_2O_2 . Others have shown that the *pyrBI* operon, which encodes aspartate carbamoyltransferase, was repressed during stringent response induced upon addition of a high concentration of valine (Turnbough, 1983). This author showed that ppGpp induction was very short and after a transient increase the molecule was degraded again. In our experiment the stringent response was probably induced right after starting irradiation and, therefore, induction of stringent response genes like *relA* and *spoT* was not observed in our analysis. What we did detect, though, were the consequences of the stringent response induction (see above). In the adapted cell population only the *pyrBI* operon was still repressed, indicating that this system was unnecessary for the survival of the adapted cells.

The biosynthesis of isoleucine (*ileS*), phenylalanine (*pheS*), histidine (*hisS*) and valine tRNA synthase (*valS*) was also upregulated after 1 h of irradiation (Table 5). Interestingly, only the phenylalanine tRNA synthase

Table 4. *Escherichia coli* genes involved in *de novo* biosynthesis of purine, pyridine and pyrimidine ribonucleotides, whose expression levels were increased by a factor ≥ 2 or decreased by a factor ≤ -2 in cultures exposed for either 1 or 50 h to UVA light in continuous culture at $D = 0.5 \text{ h}^{-1}$.

Gene no.	Name	Product and function	Induction factor	
			1 h	50 h
B0032	<i>carA</i>	Carbamoyl-phosphate synthetase, glutamine (small) subunit	-17.6	
B0033	<i>carB</i>	Carbamoyl-phosphate synthase large subunit	-7.0	
B2507	<i>guaA</i>	gmp (guanosine monophosphate) synthetase (glutamine-hydrolysing)	-2.1	
B2508	<i>guaB</i>	imp dehydrogenase (this is the first reaction unique to GMP biosynthesis)	-2.5	
B0522	<i>purK</i>	Phosphoribosylaminoimidazole carboxylase = air carboxylase, co(2)-fixing subunit	-2.2	
B4245	<i>pyrB</i>	Aspartate carbamoyltransferase, catalytic subunit	-28.2	-6.1
B1062	<i>pyrC</i>	Dihydro-orotase	-4.6	-2.3
B0945	<i>pyrD</i>	Dihydro-orotate dehydrogenase	-6.6	
B0171	<i>pyrH</i>	Uridylate kinase	-2.0	
B4244	<i>pyrI</i>	Aspartate carbamoyltransferase, regulatory subunit	-20.7	-18.9
B4246	<i>pyrL</i>	Pyrbi operon leader peptide	-4.4	-4.5
B2498	<i>upp</i>	Uracil phosphoribosyltransferase	-2.9	

Genes were only listed if the *P*-value of the three replicates was ≤ 0.05 .

Table 5. *Escherichia coli* genes involved in tRNA synthesis, whose expression levels were increased by a factor ≥ 2 or decreased by a factor ≤ -2 in cultures exposed for either 1 or 50 h to UVA light in 'LB-limited' continuous culture at $D = 0.5 \text{ h}^{-1}$.

Gene no.	Name	Product and function	Induction factor	
			1 h	50 h
B0026	<i>ileS</i>	Isoleucine tRNA synthetase	2.1	
B1714	<i>pheS</i>	Phenylalanine tRNA synthetase, alpha-subunit	2.5	5.4
B2114	<i>metG</i>	Methionine tRNA synthetase		2.0
B2518	<i>hisS</i>	Histidine tRNA synthetase	4.2	
B2791	<i>truC</i>	tRNA pseudouridine 65 synthase	-2.4	
B3166	<i>truB</i>	tRNA pseudouridine 5s synthase		2.6
B4258	<i>valS</i>	Valine tRNA synthetase	2.5	
B2392	<i>trmD</i>	trna methyltransferase, trna (guanine-7-)-methyltransferase		2.4

Genes were only listed if the *P*-value of the three replicates was ≤ 0.05 .

(*pheS*) was still upregulated in the adapted cells. In fact, Blondel and Favre (1988) showed that most tRNA species were slightly affected by UVA light but only those acylated by Phe and Pro were strongly affected *in vivo*. Both acylation levels decreased to less than 10% of their initial value during the illumination period, remained stable all along the growth lag and increased with cell mass when growth resumed. Hence, tRNA(Phe) and tRNA(Pro) are the UVA light molecular targets triggering growth delay and possibly also photoprotection and protection against UVA mutagenesis. Kramer and colleagues (1988) came to a similar conclusion stating that the inactivation of tRNA could also mediate a stringency-independent stress response possibly via adenylated dinucleotides but that also a direct interaction of cross-linked tRNA on the transcription level seems to be possible. Our results strongly support this idea as many genes induced during growth inhibition seem to be stringency-independent. Recently it was found that the biosynthesis of 4-thiouridine (s^4U) in *E. coli* tRNA requires the action of both the thiamine pathway enzyme *ThiI* and the cysteine desulfurase *IscS*. *IscS* catalyses sulfur transfer from L-cysteine to *ThiI*, which utilizes Mg-ATP to activate uridine 8 in tRNA and transfers a sulfur group to give s^4U (Lauhon *et al.*, 2004). In our experiments the *thiI* and *iscS* genes were neither differentially expressed after 1 h nor after 50 h. This indicates that the adapted cells are not *thiI* mutants like those that were isolated by others (Ramabhadran *et al.*, 1976; Kramer *et al.*, 1988). Interestingly, the gene *mgtA* encoding for an ATP-dependent Mg^{2+} transporter was strongly repressed by a factor of -16.4 in the adapted cells.

Concluding remarks

Protein turnover serves as a regulatory strategy for cells during periods of changing environmental conditions or in cases of physiological emergencies. When a crisis is encountered, the cell may require the rapid synthesis or elimination of specific gene products to allow survival.

Escherichia coli grown in continuous culture and exposed to UVA irradiation exhibits a growth inhibition with a subsequent adaptation to UVA stress. This study is the first report that uses transcriptome analysis to characterize global gene expression in *E. coli* under continuous UVA irradiation. Our analysis suggests that the adapted cells are not *thiI* mutants like they were isolated by others (Ramabhadran *et al.*, 1976; Kramer *et al.*, 1988). It was shown that indeed the stringent response is initiated, most likely via the cross-linking and inhibition of 4-thiouridine and cytidine in tRNA, but that also an additional stress response is induced including the upregulation of amino acid biosynthesis of valine, isoleucine, leucine, phenylalanine, histidine and glutamate. This induction might be due to direct inhibition of enzymes involved in the uptake or/and biosynthesis of these amino acid or results from the inhibition of N-assimilation. The reason for the enhanced biosynthesis of these amino acids can now be further investigated. Furthermore, our results corroborate earlier reports about the induction of the SOS response in UVA-irradiated cells. The induction of genes in Table 2, as for example *recA*, *recN*, *dinD*, *dinI* and *umuD*, strongly points to DNA damage as a result from UVA light exposure. Also the involvement of oxidative stress was confirmed with the induction of alkylhydroperoxidase reductase (Ahp). In the UVA light-adapted cell population we found a strong repression of the acid tolerance response. This result might imply that the cells are shutting down unneeded biosynthesis (e.g. acid resistance proteins) and can lead us to a yet unknown survival mechanism in UVA light-adapted cells. Furthermore, the role of the global stress regulator RpoS may not be as important in the adaptation of *E. coli* to UVA light-stress as it was implicated by previous results with starved cells, but it might be important for the resistance under transient conditions during exposure. The role of certain enzymes as sites of direct damage or for essential protective function (e.g. PheA, MgtA, PyrBI) can now be further investigated to elucidate their role in the stress response of *E. coli* to

UVA light. Also a comparison of the transcription profile with the proteome pattern of adapted cells and the specific measurement of RpoS and ppGpp concentrations during adaptation could give us new details about the inactivation mechanism of UVA light in enteric bacteria.

Experimental procedures

Bacterial strains

In all experiments wild-type *E. coli* K-12 MG1655 (ATCC 700926) was used.

Cultivation conditions

Used for batch cultivation was LB broth (10 g tryptone, 5 g yeast extract, 10 g NaCl per litre) (Miller, 1972) that was filter-sterilized with membrane filters (Millex GP, 0.22 µm, Millipore, Tullagreen, Ireland) and diluted to 33% volume/volume (v/v) of its original strength (unless indicated otherwise) with ultra-pure water (deionized and activated carbon-treated). Precultures were prepared for each individual experiment from the same cryo-vial stored at -80°C by streaking out the stock culture onto LB agar plates. After 15–18 h of incubation at 37°C one colony was picked, loop-inoculated into a 125 ml Erlenmeyer flask containing 20 ml of diluted LB broth and incubated at 37°C on a rotary shaker at 200 revolutions per minute. At an optical density (OD₅₄₆) between 0.1 and 0.2 [measured spectrophotometrically at 546 nm in glass (OS) cuvettes with 1 cm light path using a JASCO V550 UV/VIS spectrophotometer; JASCO, Tokyo, Japan] an aliquot of the culture was transferred into the bioreactor with 50 ml of pre-warmed diluted LB broth (5% v/v) to obtain an OD₅₄₆ of 0.002. The bioreactors for continuous culture experiments consisted of temperature-controlled and air-sparged cylindrical quartz glass tubes of 100 ml total and 50 ml working volume (WISAG, Oerlikon, Switzerland). The feed-medium consisted of 5% v/v LB broth. Before starting exposure, at least 10 volume changes were allowed for the culture to reach steady state (based on OD₅₄₆).

UVA exposure

The bioreactors were installed in a incubation device (holding up to six bioreactors) [adapted from the study by Wegelin and colleagues (1994)] equipped with a medium-pressure mercury lamp (Hanau TQ150) operated at 150 W [same wavelength spectrum as for Hanau TQ718 (Berney *et al.*, 2006b)]. The light spectra were recorded with a calibrated LI-1800 portable spectroradiometer (LI-COR, Lincoln, Nebraska, USA), 8 nm bandwidth, fitted with a model 1800-10 detector head. The lamp was placed in a cooling jacket (Duran 50 borosilicate glass) in the centre of the incubation device and the four bioreactors were arranged around the lamp at equal distance. The light emitted from the lamp passed through the glass jacket and through 35 mm of filter solution before reaching the cells in the quartz tubes. The temperature of the filter solution was maintained at 37°C and it consisted of 12.75 g l⁻¹ sodium nitrate with a cut-off at 320 nm and a half

maximum at 340 nm. The transmission property of the filter solution was checked before each experiment. Chemical actinometry with *p*-nitroanisole/pyridine was used to determine the fluence rate at the bioreactor position (Wegelin *et al.*, 1994). The light intensity applied was 50 W m⁻², which represents the average mid-day sunlight intensity in the NUV range. Throughout the experiments, OD₅₄₆ was measured as described above. After 1 h and after 50 h of exposure one UVA-exposed and one non-irradiated control bioreactor were stopped and the entire bacterial culture (50 ml) of each tube was immediately mixed with 30 g of crushed ice to cool down the samples. After centrifugation for 10 min at 15 000 r.p.m. at 4°C (Centrikon T-324, Kontron Instruments, Schlieren, Switzerland) the supernatant was discarded and total RNA was isolated (see below).

RNA isolation, synthesis of cDNA

Total RNA from *E. coli* cells was isolated with the QIAGEN RNeasy Midi Kit (QIAGEN, Basel, Switzerland) according to the manufacturer's manual. RNA in samples was quantified spectrophotometrically by measuring extinction at 260 nm and purity was checked by gel electrophoresis. Synthesis of cDNA from RNA was performed with the CyScribe First-Strand cDNA Labelling Kit (Amersham Bioscience, Little Chalfont, England). In the following description all ingredients are contained in the CyScribe kit unless otherwise noted. Reverse transcription was performed using 25 µg of total RNA (maximally in 10 µl) and 1 µl of random nonamer primers. The volume of the assay mixture was adjusted to 11 µl with RNase-free water, then the assay mixture was incubated for 5 min at 70°C, followed by incubation for 10 min at room temperature to allow the primers to anneal with the RNA. After cooling down to room temperature the reagents for the labelling reaction were added. After the addition of 4 µl of 5× CyScript buffer, 2 µl of 0.1 M DTT, 1 µl of deonucleoside triphosphate (dNTP) mixture, 1 µl of either 0.5 mM Cy3-labelled or Cy5-labelled DCTP (Amersham Bioscience), and 1 µl of CyScript reverse transcriptase (100 U µl⁻¹), the final reaction volume was 20 µl. The control cDNA (from the unirradiated bioreactor) and the probe cDNA (from either the 1 h or the 50 h irradiated bioreactor culture) were labelled differently, with the control cDNA always labelled with Cy5. Dye swapping was performed for one replicate and did not alter the result. The labelling reaction was performed at 42°C for 1.5 h, followed by RNA degradation and cDNA purification. The RNA was degraded by addition of 2 µl of 2.5 M NaOH, the mixture was then heated at 65°C for 10 min and subsequently neutralized with 10 µl of 2 M HEPES buffer. Control and probe cDNA obtained were pooled and purified on the same column of MinElute Gel Extraction Kit (QIAGEN, Basel, Switzerland) to avoid differences in extraction yields.

DNA microarray, hybridization and washing

Slide microarrays were purchased from MWG-Biotech AG (Ebersberg, Germany). The MWG *E. coli* Array contains 4288 gene-specific oligonucleotide probes representing the complete *E. coli* K12 genome. The purified cDNA was concentrated to 5 µl and was mixed with 120 µl of hybridization buffer

(MWG-Biotech AG), heated to 95°C for 3 min and cooled down on ice for 3 min. The hybridization mixture was then added to the microarray slide and covered with a coverslip. The hybridization slide was incubated overnight at 42°C. After the hybridization step the slide was washed three times, the first time for 5 min in 2× (times concentrated) SSC – 0.1% SDS, the second time for 5 min in 1× SSC, and finally for 5 min in 0.1× SSC. SSC buffer was prepared as 20× solution containing 0.3 M Na-citrate and 3 M NaCl at pH 7.0. The slides were dried by centrifugation at room temperature for 2 min at 500 g.

Image and data analysis

Microarray slides were scanned using the Affymetrix 428™ Array Scanner (High Wycombe, UK). Spot intensities and corresponding background signals were quantified with the Affymetrix Jaguar™ software version 2.0. Further data analysis was performed with the program GeneSpring from Silicon Genetics (Redwood City, CA, USA). Induction factors were calculated from the Cy3 and Cy5 signal intensities of the spot. Spots with signal intensity below a value of 50 were excluded from the analysis and the minimal IF was set to 0.01. The normalization was performed with the 50th percentile distribution of remaining spots after background correction. The mean value of the IF of a specific gene was calculated from three replicates. Biological experiments were carried out three times, which provided three biological repeats. Data from the independent experiments were combined, genes that were differentially regulated ≥ 2 and ≤ 0.5 (*t*-test, $P \leq 0.05$) were defined as being statistically significant. Induction factors of downregulated genes are displayed as $-1/IF$ for the convenience of the reader (e.g. an IF of 0.5 is displayed as -2). For two different sampling time points (1 h and 50 h) IF_1 and IF_{50} were defined as abbreviations for the IFs.

Real time PCR

cDNA was synthesized with the SuperScript reverse transcriptase kit (Invitrogen, Basel, Switzerland) according to the manufacturer. After cDNA synthesis the total volume was adjusted to 40 μ l before starting real time PCR. Real time PCR was conducted according to the manufacturer with SYBR® Green PCR Master Mix (Applied Biosystems, Rotkreuz, Switzerland). Primers (Microsynth, Balgach, Switzerland) were designed with the software Primer Express® v2.0 (Applied Biosystems) for eight genes (Appendix S2). Primer optimization was performed. The real time PCR was conducted using ABI Prism® 7000 (Applied Biosystems). The results were normalized using the gene *rrsB* as endogenous control (Table 6). Real time PCR data were analysed with ABI Prism® 7000 SDS software version 1.0 according to the System User Bulletin No. 2 Relative Quantitation of Gene Expression (P/N 4303859) from Applied Biosystems.

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Table 6. Comparison of mRNA expression levels of selected genes measured with either the microarray or the real-time PCR method after 50 h of UVA irradiation.

Gene no.	Name	Induction factor	
		Microarray	RT-PCR
B3942	<i>katG</i>	1.9	2.9
B0605	<i>ahpC</i>	2.2	3.3
B2699	<i>recA</i>	6.7	3.7
B2573	<i>rpoE</i>	-1.1	-1.9
B0417	<i>thil</i>	-1.2	-1.2
B1749	<i>xthA</i>	-1.4	-1.2
B2741	<i>rpoS</i>	-1.1	-1.8
B1732	<i>katE</i>	-2.3	-3.3

Only genes with *P*-values ≤ 0.05 were selected.

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Supplementary material

The following supplementary material is available for this article online:

Appendix S1. *Escherichia coli* genes whose expression levels were increased by a factor ≥ 2 or decreased by a factor ≤ -2 in cultures exposed for either 1 or 50 h to UVA light in 'LB-limited' continuous culture at $D = 0.5 \text{ h}^{-1}$. Genes were only listed if the *P*-value of the three replicates was ≤ 0.05 .

Appendix S2. Primers used in the real time PCR experiments.

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