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# Amino Acid Deprivation-induced Stringent Response and Dps Confer Added Levels of Protection from UV Radiation in *Escherichia coli*

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**Nutrient-starved bacteria undergo physiological changes that increase bacterial resistance to nutrient and oxidative stresses, chemical and metal toxicity, and radiation, resulting in continued survival. Previous studies have demonstrated that cells in stationary phase are more resistant to UV radiation than cells in the exponential growth phase. The DNA-binding protein, Dps, plays a key role in surviving UV radiation and other stresses in stationary phase. This study investigated the role of Dps in resistance to UV irradiation during stringent response in Escherichia coli ZK126 and ZK1146, a *dps* mutant. We report that Dps-associated resistance to UV irradiation is present not only during stationary phase, but may also be present in exponential phase and stringent response. However, there is evidence to suggest that the activation of the stringent response confers added protection in *E. coli* to UV radiation in a Dps-independent manner.**

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Adaptation to amino acid starvation is known as the stringent response and is induced via increased guanosine tetraphosphate (ppGpp) levels. During amino acid starvation, levels of the cognate uncharged tRNA increase. When uncharged tRNA molecules bind to the ribosomal A site, the ribosome-associated molecule RelA catalyzes the synthesis of (p)ppGpp (19) which accumulates resulting in the induction of many stress-resistant genes including the DNA binding protein, *dps* (2).

In stationary phase, Dps has a well-characterized role in resistance to oxidative stresses through up-regulation by the stationary phase transcription factor, RpoS, and stabilization by ClpA and ClpP chaperone activity. ClpA and ClpP also appear to be involved in Dps synthesis at the translational level. Dps levels are regulated during exponential phase through OxyR, $\sigma^{70}$ and the ClpXP (3,16). OxyR and $\sigma^{70}$ are involved in *dps* gene expression and ClpXP degrades the Dps protein. Antagonistic action of these two processes maintains levels at 6,000 molecules per cell during exponential phase.

Dps confers stress resistance by sequestering DNA, binding metals, and regulating the expression of several stress response genes (12). In addition, Dps possesses ferroxidase activity. These features all play a role in the protection of the chromosome from oxidative damage (9). Dps plays an especially important protective role in stationary phase: During transition to stationary phase in *E. coli*, Dps becomes one of the most abundant proteins reaching up to 200,000 molecules per cell (4). Dps binds the chromosome forming a highly stable nucleoprotein complex known as a biocrystal. Sequestration of DNA in this Dps biocrystal protects the chromosome from various DNA-damaging assaults (18). In stationary phase, Dps has been shown to protect *E. coli* from oxidative stress, UV and gamma irradiation, copper and iron toxicity, acid/base shock, fatty acid starvation, and general oxidative stress from reactive oxygen species (12).

UV radiation generates oxidative radicals and creates single and double stranded DNA breaks. Dps acts as a substitutive substrate to both UV radiation and oxidative radicals thus conferring UV resistance during stationary phase. Dps is maintained at basal levels in *E. coli* growing in exponential phase.

More thorough knowledge about the role of Dps in exponential phase cells and in the amino acid deprivation-induced stringent response is required. More specifically, the role of the added levels of Dps accumulated during the stringent response is yet to be elucidated. The goal of this study was to elaborate on the role of Dps in *E.coli* UV protection. Previous studies have shown that stationary phase cells are more resistant to UV radiation than log-phase cells (1). Moreover, Pang *et al.* presented a model where Dps was the primary protein responsible for increased survival in stationary cells (13). Nair *et al.* previously confirmed this by showing that *dps* mutant *E. coli* were more sensitive to UV irradiation than wild-type *E. coli* during stationary phase (12). Our results indicate that Dps protects cells from UV irradiation during exponential phase and stationary phase. Our results also indicate that activation of the stringent response does increase UV resistance in *E. coli*, but this may be in a Dps-independent manner.

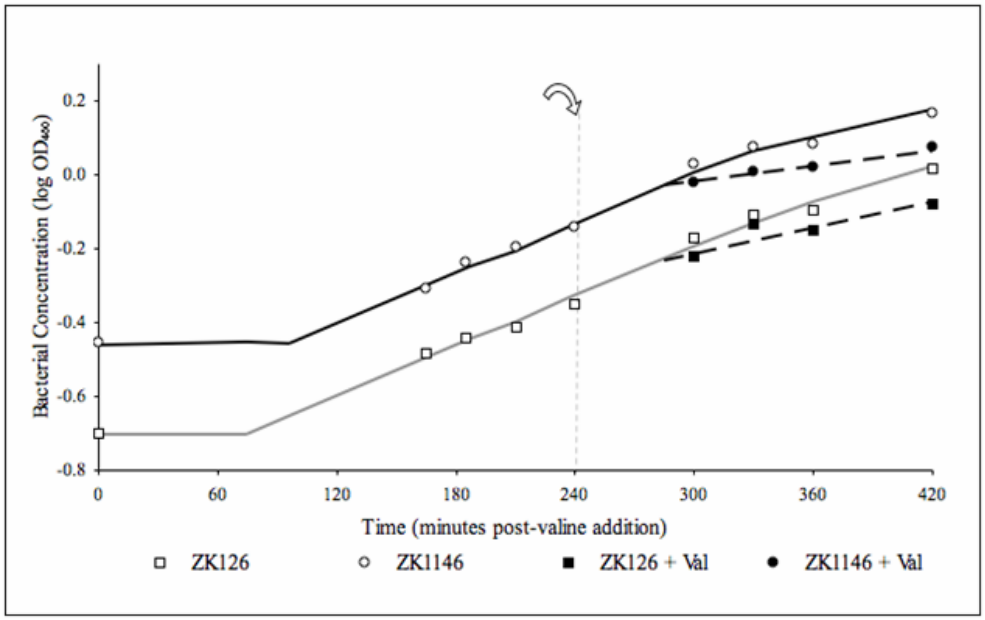


**Fig. 1 - Growth Characteristics of *E. coli* ZK126 and *E. coli* ZK1146 in Exponential Phase or Stringent Response.** Wild-type (ZK126) and *dps* mutant (ZK1146) *E. coli* were grown aerobically in M9 minimal media at 37ºC, 250 rpm after inoculation from overnight cultures in the same media at time 0. Valine was added at 240 minutes, as indicated by the arrow. Wild-type *E. coli* in exponential phase (ZK126), wild-type *E. coli* in stringent response (ZK126 + Val), *dps* mutants in exponential phase (ZK1146), and *dps* mutants in stringent response (ZK1146 + Val)

## MATERIALS AND METHODS

**Bacterial strains.** *E. coli* strains ZK126 [W3110Δ*lac*U16tna2] (isogenic wild-type control) and *E. coli* ZK1146 [ZK126dps::cam] (*dps* mutant) were graciously donated by the Kolter laboratory, Department of Microbiology and Molecular Genetics, Harvard Medical School.

**Media, reagents and treatments**. Cultures were grown in M9 minimal liquid medium (14) supplemented with 0.1% glycerol and 50 µg/mL thiamine (Sigma T4625, 2 mg/mL stock). Treatment with valine (Sigma V0500, 6 mg/mL stock) was at a final concentration of 60 µg/mL in Experiment 1 or 120 µg/mL in Experiment 2, where 0 µg/mL was used as a negative control in both experiments. Culture samples were spread-plated on to LB nutrient agar (10 g/L tryptone, 5 g/L NaCl, 5 g/L yeast extract, 15g/L agar) and irradiated at either 2.5 mJ/cm$^2$ in Experiment 1 or 4.0 mJ/cm$^2$ in Experiment 2 using the UV Stratalinker ® Model 2400 (Stratagene, 40071), where 0 mJ/cm$^2$ was used as a negative control for both experiments. Radioactive $^{14}$C-uracil (Sigma, U3879) was added at a concentration of 0.333 µCi/mL to incorporation samples. Additional cold uracil (Sigma, U0570) was added to incorporation samples at a final concentration of 2.5 µg/mL.

**Culturing Methods.** Supplied samples of *E. coli* ZK126 and *E. coli* ZK1146 were streaked onto LB plates to assess purity. Plates were incubated overnight at 37ºC and isolated colonies were used to inoculate overnight cultures in M9-broth for all subsequent experiments. Strains were re-inoculated from overnight cultures to an $OD_{460}$ of 0.2 to 0.4 and grown to early-log phase before the addition of valine. Growth and UV sensitivity were monitored in a shaking water-bath at 37ºC for up to 24 hours.

**Determination of Growth Characteristics.** Growth curves were constructed by measuring $OD_{460}$ of a growing culture re-inoculated from an overnight culture with a DU® 800 Beckman Coulter UV/Vis spectrophotometer in each of the four treatment groups over a 24-hour time course. Treatment groups were *E. coli* ZK126, ZK126 + valine, ZK1146 *dps* mutant and ZK1146 + valine.

**Uracil Incorporation**. [$^{14}$C]-labelled uracil and additional cold uracil were added to 2.0 mL of each of the four treatment cultures at concentrations indicated above, concurrent with valine addition. These 2.0 mL cultures were grown at 37º C in 10-mL tubes and sampled in duplicate at varying times for the first 2 hours. The procedure for [$^{14}$C]-labelled uracil incorporation analysis was as described previously (14) and was only completed for Experiment 1 samples to verify potential presence of a stringent response.

**Survival Assay.** The four treatment groups were grown in 25-mL cultures and sampled at varying time-points post-valine addition. Fifty micro-liters of media were removed, diluted ($5x10^{-6}$, $1x10^{-6}$, and $2x10^{-7}$) with saline and spread onto LB plates. The samples were immediately subjected to UV radiation (without a lid) in the UV Stratalinker with doses as listed earlier. Plates were grown overnight at 37ºC and percentage survival was calculated from the ratio of colony forming units that survived irradiation as compared to non-irradiated controls.

## RESULTS

**Growth characteristics of *E. coli* ZK126 and *E. coli* ZK1146.** Wild-type (ZK126) and *dps* mutant (ZK1146) *E. coli* showed lag times of approximately 75 and 95 and minutes, respectively (Fig. 1) after re-inoculation from an overnight culture. Valine was added when cells were in early log phase at 240 minutes. A reduction in the growth rate was observed in valine-treated cells relative to untreated cells after 30 minutes. Cultures of untreated wild-type cells reached stationary phase and saturated at 1.5 x $10^8$ cells/mL while valine-treated cultures stopped at only 7.9 x $10^7$ cells/mL (Fig. 2). Similarly, cultures of untreated *dps* mutant cells saturated at 2.0 x $10^8$ cells/mL while valine-treated cultures stopped at only 1.2 x $10^8$

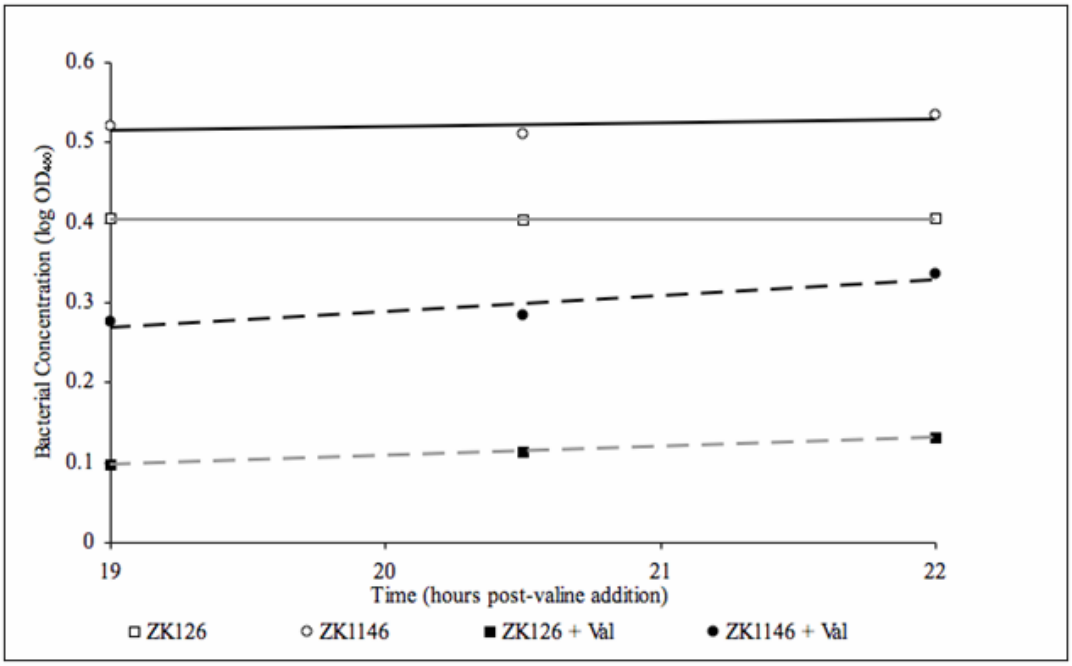


**Fig. 2 - Growth Characteristics of *E. coli* ZK126 and *E. coli* ZK1146 in Stationary Phase or Stringent Response.** Wild-type *E. coli* in stationary phase (ZK126), wild-type *E. coli* in stringent response (ZK126 + Val), *dps* mutants in stationary phase (ZK1146), and *dps* mutants in stringent response (ZK1146 + Val) were grown aerobically in M9 minimal media at 37ºC. Stringent response was induced at 240 min and at these later time points (19 to 22 hours post-valine addition), untreated cell cultures (ZK126 & ZK1146) have entered stationary phase and treated cell cultures (ZK126 + Val & ZK1146 + Val) are still under stringent response.

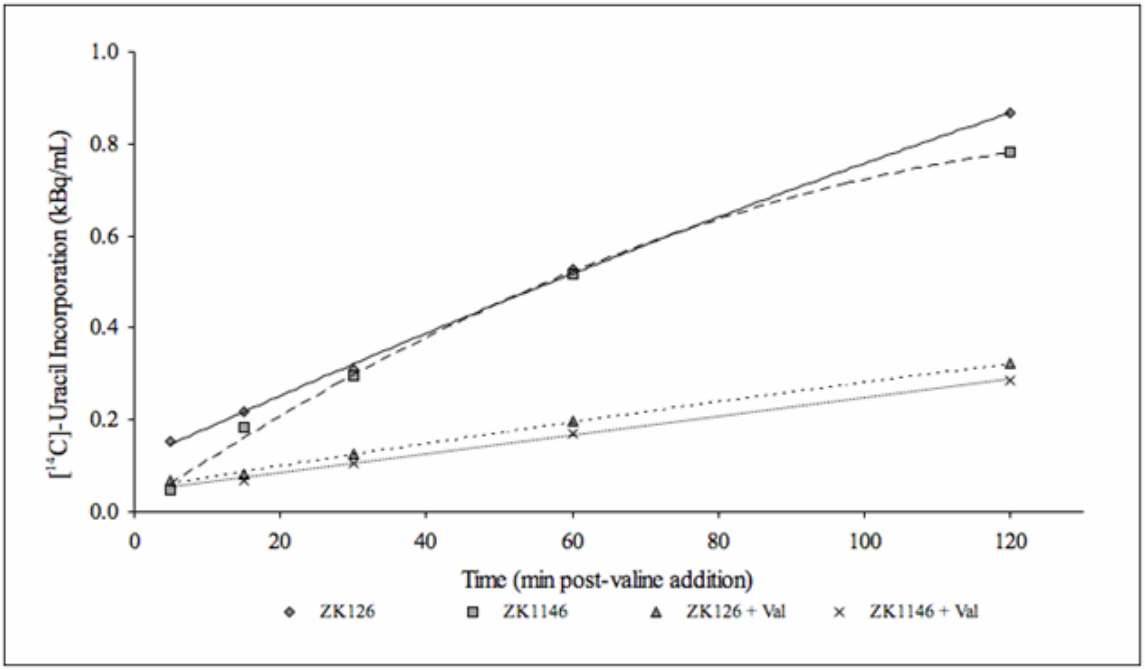


**Fig. 3 - Valine Decreases Transcriptional Activity and Induces Stringent Response in *E. coli* ZK126 and *E. coli* ZK1146**. Stringent response induction by valine in wild-type (ZK126) and *dps* mutant (ZK1146) *E. coli* was measured by uracil incorporation. A dose of 60 µg/ml L-valine was added at 0 minutes and cells were grown aerobically in M9 minimal media at 37ºC.

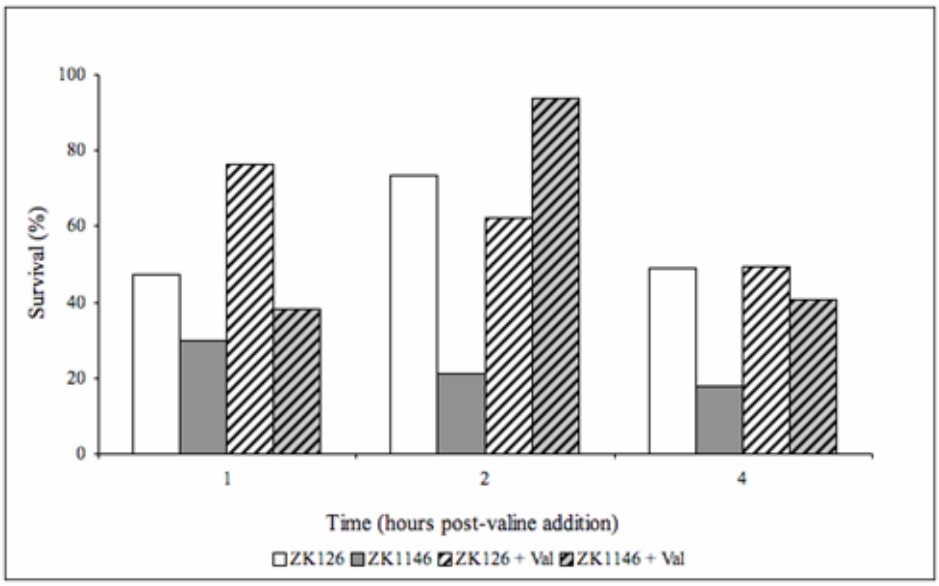


**Fig. 4 – UV Resistance in *E. coli* ZK126 and *E. coli* ZK1146 in Exponential Phase or Stringent Response Survival Assay.** *Experiment 1.* Stringent response was induced by valine addition (60 µg/mL). Wild-type *E. coli* in exponential phase (ZK126) or in stringent response (ZK126 + Val) and *dps* mutants in exponential phase (ZK1146), or in stringent response (ZK1146 + Val) were plated on LB agar plates and subjected to UV dose of 2.5 mJ/cm$^2$.

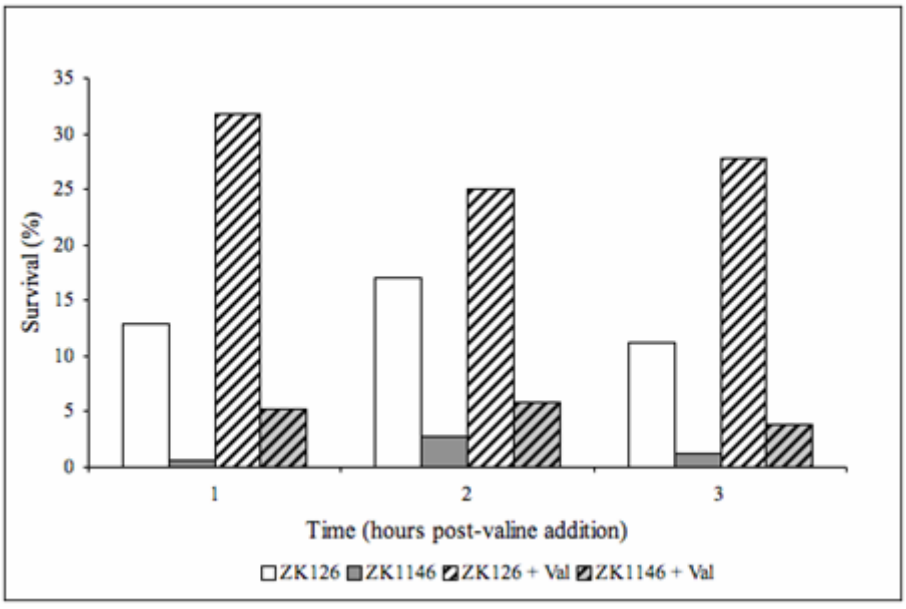


**Fig. 5 - UV Resistance in *E. coli* ZK126 and *E. coli* ZK1146 in Exponential Phase or Stringent Response.** *Experiment 2.* Stringent response was induced by valine addition (120 µg/mL). Wild-type *E. coli* in exponential phase (ZK126) or in stringent response (ZK126 + Val) and *dps* mutants in exponential phase (ZK1146), or in stringent response (ZK1146 + Val) were plated on LB agar plates and subjected to UV dose of 4.0 mJ/cm$^2$.

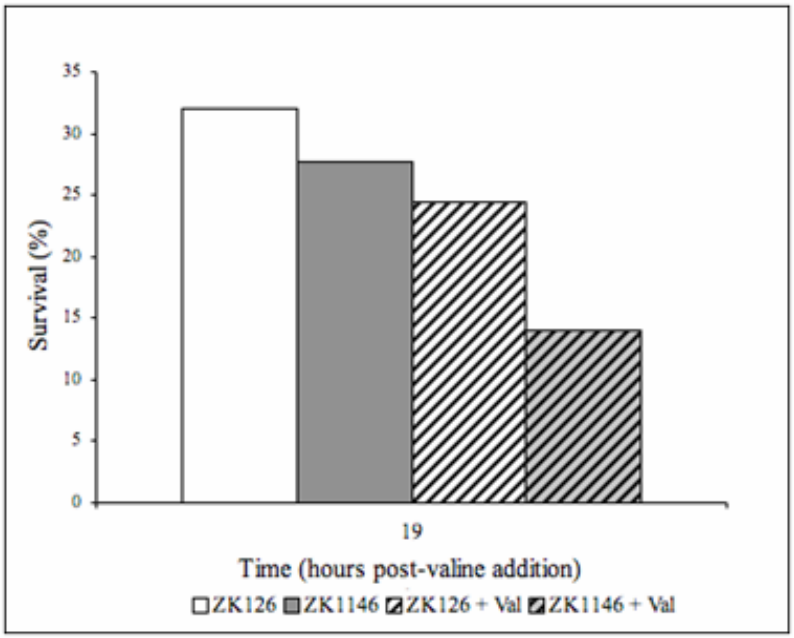


**Fig. 6 - UV Resistance in *E. coli* ZK126 and *E. coli* ZK1146 in Stationary Phase or Stringent Response.** *Experiment 2.* Wild-type *E. coli* in stationary phase (ZK126) or in stringent response (ZK126 + Val) and *dps* mutants in stationary phase (ZK1146) or stringent response (ZK1146 + Val) were plated on LB agar plates and subjected to UV dose of 4.0 mJ/cm$^2$.

cells/mL (Fig. 2). From preliminary growth data and subsequent plating, it seems that the *dps* mutant cells are three percent larger in size than our parental strain, ZK126 (data not included).

**Inability to induce a full stringent response**. As can be seen in Fig. 3, a dosage of 60 µg/mL valine yields a 3-fold decrease in uracil incorporation for both strains. This is indicative of the inhibited transcription levels characteristic of stringent response, however, we see that there is some residual uracil incorporation: Stringent response was not fully established at this dosage. Controls performed to verify total radioactive material present in the system showed no change between the beginning and the end of the experiment (data not included).

**Role of Dps in UV resistance during stringent response**. At a UV dosage of 2.5 mJ/cm$^2$ at the log phase wild-type cells had greater UV resistance than *dps* mutants at all three sampled times (Fig. 4), an expected and established phenotype. Neither strain showed significant or consistent valine-induced patterns of UV resistance between selected time-points at this dosage. At a UV dosage of 4 mJ/cm$^2$ were more ideal (Fig. 5). All three sample times showed that log-phase wild-type cells had greater than 7 times the UV resistance of *dps* mutant cells. Further, results show that valine-treated wild-type and *dps* mutants were on average, 2 and 5 times more resistant to UV radiation than untreated cells. Since time may be a factor, it should be noted that—of the time-points tested—relative UV resistances between treated and untreated was the lowest for both cell strains at1 hour. Wild-type valine-treated cells had the greatest survival rate throughout the first three hours after addition. Untreated wild-type followed by treated *dps* mutants displayed the next greatest survival rates and finally, untreated *dps* mutants had the lowest survival rate. Differing time-points seem to be optimal for UV resistance in different strains, but no discernible pattern could be seen.

**Role of Dps in UV resistance during stationary phase.** At UV dosage of 4 mJ/cm$^2$, cells sampled at 19 hours post-valine addition showed both untreated wild-type and *dps* mutants had greater UV resistance than treated cell strains (Fig.6). Further, wild-type *E. coli* had greater UV resistance than *dps* mutants with or without valine treatment.

**Induction of increased UV resistance in *E. coli* during stringent response.** Wild-type cells in log phase displayed an average survival rate of 14% (Table 1). Stringent response elicited 19%, 12%, and 15% increases in survival rate at 1, 2 and 3 hours, respectively. *E. coli dps* mutants growing in log-phase had an average survival rate of 1.5% where stringent response elicited 5%, 3%, and 3% increases of UV resistance in these Dps mutants, again at 1, 2 and 3 hours.

## DISCUSSION

**Table I: Calculation of Absolute Survival in Response to UV Radiation in wild-type (ZK126) vs *dps* mutant (ZK1146) *E. coli*, when Induced to Enter Stringent Response**

| | *dps* Mutants | | | Wild-type | | | |
|---|---|---|---|---|---|---|---|
| Time (hours) | Baseline survival (%) | Stringent Response survival (%) | Survival due to "other" Stringent response factors (%) | Baseline survival (%) | Stringent Response survival (%) | Total survival due to stringent response (%) | Survival due to increased Dps levels induced by stringent Response (%) |
| 1 | 1 | 5 | 5 | 13 | 32 | 19 | 14 |
| 2 | 3 | 6 | 3 | 17 | 25 | 8 | 5 |
| 4 | 1 | 4 | 3 | 11 | 28 | 17 | 14 |

Growth rates of *E. coli* ZK126 (wild type) and *E. coli* ZK1146 (*dps* mutant strain) were significantly hindered within 30 minutes of valine treatment, at 60 μg/mL in Experiment 1 (data not shown) and even more so at 120 μg/mL (Fig 1). Valine was added to induce isoleucine deprivation (6); absence of these amino acids results in the stringent response (12). At the low dosage of valine (Fig. 4), to verify that induction took place, relative [$^{14}$C]-uracil incorporation levels were measured as an indicator of stable RNA biosynthesis shutdown, a key characteristic of the stringent response (5). This dosage of valine was able to decrease the transcriptional activity of both wild-type and mutant cells (Fig. 3), however residual transcriptional activity was seen, suggesting that the stringent response was not in full effect. In order to correct this, the valine concentration was doubled for the second experiment and while [$^{14}$C]-uracil incorporation levels were not examined a second time, it is presumed and supported by growth data that the stringent response was more robust under these conditions (Fig. 1).

Despite these issues, we see a clear protective relationship of Dps for UV resistance in *E. coli*, as is evident in survival patterns for cells exposed at either UV dose (Fig. 4 and Fig. 5). In our system, presence of Dps in wild-type cells conferred a survival advantage over *dps* mutants in exponential phase, stringent response (when fully established), and stationary phase.

At low dose of valine (Fig. 4), survival rates were very high; often at 70%, drawing doubt to the toxicity of the UV dose (2.5 mJ/cm$^2$) and affecting the significance of the numbers found. This, along with the observation that the valine dose used did not activate a full stringent response, compelled us to perform the second experiment at a greater UV dose of 4.0 mJ/cm$^2$ and a valine dose of 120 μg/mL for a greater degree of stringent response and greater killing.

In the second experiment, wild-type cells consistently displayed a significant survival advantage over *dps* mutants when exposed to UV stress. Presence of Dps increased the survival rate of cells in exponential phase 13-fold on average (Fig. 5). Dps increased the survival of cells in stringent response 6-fold on average (Fig. 5). These results clearly show that Dps is a significant contributor to UV resistance in both exponential phase and stringent response in *E. coli*.

Activation of the stringent response conferred increases in UV resistance in both wild-type and *dps* mutant strains. At the low dosage of valine (Fig. 4), the stringent response conferred a survival advantage in wild-type cells at only 1-hour post-valine addition. The stringent response is involved in increasing the survival rate of *dps* mutants for up to at least 3 hours post-valine addition. These survival rates in the mutants approach and at times exceed the survival rates of wild type cells in exponential phase (Fig. 4). This suggests that the stringent response alone is able to confer an added degree of UV resistance in *dps* mutants. Furthermore, it appears that Dps levels in exponential phase are often sufficient to compensate for the lack of this added degree of resistance in wild-type cells. Results from this experiment, however, are drawn into question due to incomplete activation of the stringent response (Fig. 3) and the questionable toxicity of the UV dose. Therefore, more weight will be placed on data with a higher dosage of valine and UV.

At the higher dosage of valine (Fig. 5), wild-type and *dps* mutant cells both showed increases in UV resistance upon entering stringent response from exponential phase (2.5 and 3-fold increases, respectively), however, wild-type cells still had an overall survival advantage over *dps* mutants. Therefore, it is evident that the stringent response is involved in giving cells an increased level of UV resistance. The similar degree of increase in UV resistance between wild-type and *dps* mutant *E. coli* in stringent response suggests that this increase may be Dps-independent –i.e. this added resistance a) may be due to increased Dps effectiveness in conferring UV

**Table II: Calculation of Fold Change in Survival in Response to UV Radiation, of wild-type (ZK126) vs *dps* mutant (ZK1146) *E. coli*, When Induced to Enter Stringent Response**

| | *dps* Mutants | | | Wild-Type | | |
|---|---|---|---|---|---|---|
| Time | Baseline survival (%) | Stringent Response Survival (%) | Fold Change | Baseline survival (%) | Stringent Response Survival (%) | Fold Change |
| 1 | 1 | 5 | 9 | 13 | 32 | 2 |
| 2 | 3 | 6 | 2 | 17 | 25 | 2 |
| 3 | 1 | 4 | 3 | 11 | 28 | 3 |

resistance (from increased levels of the protein increased Dps:DNA complexing), but other factors also make contributions that are overshadowed by the role of Dps or b) does not involve Dps: Instead, Dps is involved in providing a higher level of UV resistance in general. The latter explanation suggests that other mechanisms such as RecA DNA repair enzyme or presence of a higher genome copy number are involved in the added UV resistance of *E. coli* cells in stringent response.

To resolve the perplexing issue of relative contributions of Dps and Dps-independent factors in stringent response towards UV resistance, the absolute increases and differences in UV resistance between the treatment groups were analyzed (Table I). It is again evident from this analysis that the stringent response provides an increased level of UV resistance and factors other than Dps contribute to UV resistance in cells in stringent response. The greater absolute increases in UV resistance in wild-type cells relative to *dps* mutants suggests that Dps may be more important in conferring this greater degree of UV resistance increase in stringent response. The added levels of Dps (increase over basal Dps levels present in log-phase cells) may be responsible for 14%, 6%, and 14% of the stringent response-associated increase in UV resistance (Table I) at 1,2 and 3 hours after valine addition. This analysis is based on absolute increases in UV resistance. However, the significant difference in baseline survival in exponential phase, between the wild-type and *dps* mutant strains suggests that the comparison of absolute values may not be a fair - i.e. the mutants have such a low baseline survival, that it may not be expected that they can ever truly compete with the wild-type cells, even in generating the increase of UV resistance associated with stringent response. Therefore, the analysis of absolute differences in survival is inconclusive.

The average increase of survival rate in stringent response relative to the survival rate in exponential phase is similar in both wild-type and *dps* mutant cells (Table II). Wild-type *E. coli* exhibit an average increase in UV resistance of 2.5-fold when entering stringent response from exponential phase; this increase is 3-fold in *dps* mutants. These observations suggest that the added degree of UV resistance associated with stringent response may be Dps-independent.

In the experiement at low dosage of valine (Fig. 4), apparent culture contamination disqualified stationary phase results, so in order circumvent these issues, precautions were taken to remove this risk in Experiment 2. At 19 hours, wild-type and *dps* mutant strains show similar levels of UV resistance (Fig. 6). Although cells in stringent response have been under nutrient stress for an extended period of time at later time points, they do not enter stationary phase. Stringent response involves the shutdown of ribosome synthesis resulting in significant decreases in the overall rate of protein translation (5) where a shift to stationary phase requires the synthesis of numerous proteins (e.g. RpoS) specific to surviving nutrient limitation. Wild-type cells exhibit only a 4% survival advantage over *dps* mutants (Fig. 6). Cells entering stationary phase from exponential phase are observed to have a noticeable survival advantage over cells in stringent response (Fig. 6). This trend is seen in both wild-type and *dps* mutant cells. These results suggest that the mechanisms activated in stationary phase are superior to those in exponential phase or stringent response in conferring UV resistance. Further, our results suggest that although Dps is the most prevalent nucleoid protein in stationary phase, it may not be the most important factor in UV resistance in this growth phase, which is in direct contrast to previous findings from Nair and Finkel (12). However, Nair and Finkel used a substantially higher dose of UV radiation to exhibit the differences in UV survival between wild-type and *dps* mutant cells in stationary phase. We may have to increase the UV dose substantially in our experiment to observe significant disparities in survival rate between the two types of cells in stationary phase. Overall, it is clear that cells in stationary phase have a UV survival advantage over cells in exponential phase and the stringent response.

Evidence showing that amino acid deprivation induces an increase in *E. coli* stress resistance via Dps has widespread implications. Dps is present in and has homologues in many other bacterial species, providing resistance to multiple stresses. Since most bacteria lead a feast or famine lifestyle, the robustness gained due to Dps and other stress resistance-associated molecules during starvation conditions have major implications on biotechnology, bacterial culturing, and human health.

Basal levels of Dps maintained in exponential phase provide substantial levels of UV resistance in *E. coli* cells. Amino acid deprivation-induced stringent response confers added UV resistance in the absence of Dps, while its presence, this relative effect is enhanced. Dps does not appear to be the basis of stringent response-associated increases in UV resistance, but contributes to the overall UV resistance of *E. coli* in stringent response. The levels of UV resistance reached in stringent response are lower than those observed in stationary phase.

## FUTURE EXPERIMENTS

It is evident that Dps is not the only factor responsible for conferring UV resistance in the stringent response, as seen by the significant survival increases in the *dps* mutant strain (Fig. 5). To determine the relative contribution of Dps as compared to other protective factors during the stringent response, this experiment could be repeated by comparing survival in these *dps* mutants to other mutants for proteins known to be important in UV resistance. These knockouts should be generated from the same wild-type ZK126 *E. coli* parent strain. Further studies could be performed to determine whether other factors that protect against UV radiation during stringent response or stationary phase also protect by binding the chromosome and, if so whether it is by a similar compaction and sequestration scheme.

Added information on Dps levels present throughout our experiment would be useful to quantitate: we recommend doing so by lysing cells at certain time-points after induction of stringent response and isolating protein for Western Blots with an anti-Dps-antibody (18). Quantitating translation would elucidate whether there is an increased production of Dps, increased stability or increased complexing of Dps with DNA for the added protection seen in this study.

If a relaxed strain can be made, or if the strains used here were treated with a combination of both valine and chloramphenicol to uncouple stringent response from starvation, future experimenters could determine whether survival is ppGpp based.

Finally, to rule out the confounding factor of resistance based on multiple copies of the chromosomes present, future studies could quantify DNA during stringent response as compared to logarithmic phase cells.

## ACKNOWLEDGEMENTS

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