Role of *Escherichia coli* RpoS, LexA and H-NS global regulators in metabolism and survival under aerobic, phosphate-starvation conditions

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It has been suggested that *Escherichia coli* can resist aerobic, glucose-starvation conditions by switching rapidly from an aerobic to a fermentative metabolism, thereby preventing the production by the respiratory chain of reactive oxygen species (ROS) that can damage cellular constituents. In contrast, it has been reported that *E. coli* cannot resist aerobic, phosphate (Pᵢ)-starvation conditions, probably because of the maintenance of an aerobic metabolism and the continuous production of ROS. This paper presents evidence that *E. coli* cells starved for Pᵢ under aerobic conditions indeed maintain an active aerobic metabolism for about 3 d, which allows the complete degradation of exogenous nutrients such as arginine (metabolized probably to putrescine via the SpeA-initiated pathway) and glucose (metabolized notably to acetate), but cell viability is not significantly affected because of the protection afforded against ROS through the expression of the RpoS and LexA regulons. The involvement of the LexA-controlled RuvAB and RecA proteins with the RecG and RecBCD proteins in metabolism and cell viability implies that DNA double-strand breaks (DSB), and thus hydroxyl radicals that normally generate this type of damage, are produced in Pi-starved cells. It is shown that induction of the LexA regulon, which helps protect Pi-starved cells, is totally prevented by introduction of a recB mutation, which indicates that DSB are actually the main DNA lesion generated in Pi-starved cells. The requirement of RpoS for survival of cells starved for Pi may thus be explained by the role played by various RpoS-controlled gene products such as KatE, KatG and Dps in the protection of DNA against ROS. In the same light, the degradation of arginine and threonine may be accounted for by the synthesis of polyamines (putrescine and spermidine) that protect nucleic acids from ROS. Besides LexA and RpoS, a third global regulator, the nucleoid-associated protein H-NS, is also shown to play a key role in Pi-starved cells. Through a modulation of the metabolism during Pi starvation, H-NS may perform two complementary tasks: it helps maintain a rapid metabolism of glucose and arginine, probably by favouring the activity of aerobic enzymes such as the NAD-dependent pyruvate dehydrogenase complex, and it may enhance the cellular defences against ROS which are then produced by increasing RpoS activity via the synthesis of acetate and presumably homoserine lactone.

**Keywords:** H-NS, LexA, phosphate starvation, reactive oxygen species, RpoS

**Abbreviations:** DSB, double-strand breaks; PDH, pyruvate dehydrogenase complex; ROS, reactive oxygen species.


**INTRODUCTION**

*Escherichia coli* is a facultative anaerobe which is able to obtain energy through aerobic respiration, anaerobic respiration, or fermentation. During the exponential phase of growth under aerobic conditions, aerobic respiration is preferred to anaerobic respiration or fermentation because it is the most energetically favourable process (Guest, 1992; Gennis & Stewart, 1996). Such a preference for oxygen as terminal electron acceptor has an inherent drawback: the generation by the respiratory chain of the toxic superoxide anion radical \( \text{O}_2^- \). Superoxide and the resulting other reactive oxygen species (ROS) such as hydrogen peroxide \( \text{H}_2\text{O}_2 \) and the hydroxyl radical \( \text{HO}^- \) can damage all cellular components, lipids, proteins and nucleic acids (González-Flecha & Demple, 1995; Lynch & Lin, 1996; Vaughan, 1997). In the case of DNA oxidation, it has been suggested that iron released from dehydratases by superoxide, and adventitiously deposited on the surface of DNA, can catalyse the conversion of \( \text{H}_2\text{O}_2 \) into hydroxyl radicals that readily attack the adjacent sugar and base moieties (Keyer & Imlay, 1996). Damage of DNA bases produces a wide variety of alterations that can give rise to base-substitution mutations following erroneous replication of modified bases (Henle & Linn, 1997; Kreutzer & Essigmann, 1998). Damage of DNA sugar generates essentially single-strand breaks (Henle & Linn, 1997) that can produce potentially lethal double-strand breaks (DSB) after collapsing the replication fork (Asai et al., 1994; Cox, 1997). DSB can also result from the chance occurrence of overlapping single-strand breaks in the complementary strands of a non-replicating DNA molecule (Rupp, 1996; Chol Ha et al., 1998; Henle et al., 1999).

Cells are efficiently protected against ROS attack by an array of protective mechanisms. For example, *E. coli* possesses superoxide dismutases \( \text{SodA}, \text{SodB} \), catalases \( \text{KatE}, \text{KatG} \), peroxidases \( \text{AhpCF}, \text{Tpx} \), DNA-protecting compounds \( \text{Dps}, \text{polyamines} \), and DNA-repair enzymes specific for either oxidized bases \( \text{XthA}, \text{Nfo}, \text{Nth} \) and MutMTY for oxidized purines) or DSB \( \text{RecBCD}, \text{RecA}, \text{RecG}, \text{RuvABC} \) \( \text{Dempel} \) & \text{Harrison}, 1994; Lynch & Lin, 1996; Rupp, 1996; Henle & Linn, 1997; Chol Ha et al., 1998). Many of the genes implicated in the defence against ROS are inducible and belong to regulons that enable bacteria to cope with various stresses: the SoxRS regulon, including \text{sodA} and \text{nfo}, which responds to redox-cycling drugs; the OxyR regulon, including \text{katG} and \text{dps}, which is induced in actively growing cells in response to \( \text{H}_2\text{O}_2 \)-mediated oxidation; the RpoS \( \sigma^S \) regulon, including also \text{katG} and \text{dps}, which is induced when bacteria enter stationary phase; and the LexA regulon, including \text{recA} and \text{rurAB}, which is induced by DNA damage (Sak et al., 1989; Altuvia et al., 1994; Hengghe-Aronis, 1996; Walker, 1996; González-Flecha & Demple, 1997; Gort & Imlay, 1998).

Recent data suggest that, under starvation conditions, *E. coli* can abandon its normal preference for aerobic metabolism in order to avoid the production of ROS. Indeed, when bacteria grown under aerobic conditions are starved for glucose, the pattern of protein synthesis is immediately changed in a manner that is reminiscent of a shift from aerobicism to anaerobiosis (Nyström, 1994). For example, fermentative enzymes such as the pyruvate formate-lyase \( \text{Pfl} \) (Böck & Sawers, 1996) are produced in increased amounts, whereas aerobic enzymes such as the NAD-dependent lipoamide dehydrogenase subunit \( \text{Lpd} \) present notably in the pyruvate dehydrogenase complex \( \text{PDH} \) (Quail et al., 1994) are produced in decreased amounts (Nyström, 1994). These changes in protein synthesis are thought to help *E. coli* to survive prolonged starvation (Nyström et al., 1996). Two lines of evidence suggest, however, that *E. coli* cells starved for phosphate \( \text{P}_i \) maintain an aerobic metabolism. First, at the onset of \( \text{P}_i \) starvation, the rate of synthesis of the AceF subunit of the aerobic enzyme PDH \( \text{AceEF-Ldp} \) is not significantly affected, while the rate of synthesis of the fermentative enzyme \( \text{Pfl} \) is strongly reduced (VanBogelen et al., 1996). Second, Davis et al. (1986) have shown that *E. coli* strain D10 could not survive under aerobic \( \text{P}_i \)-starvation conditions (viability was reduced to \( 10^{-5} \) by about 3 d of incubation) because of an extensive degradation of ribosomes. Nyström et al. (1996) have suggested that such an unrestrained degradation of ribosomes could result from an unchecked respiratory activity generating high levels of ROS. The possibility that *E. coli* cells starved for \( \text{P}_i \) may suffer more oxidative damage than cells starved for glucose could help explain our previous finding that DNA-repair genes that belong to the LexA regulon are induced when cells are starved for \( \text{P}_i \), but not when they are starved for glucose (Dri & Moreau, 1993). For unknown reasons, induction of the LexA regulon is then dependent upon the nucleoid-associated protein H-NS (Dri & Moreau, 1993), which normally helps *E. coli* to adapt to stressful environmental conditions (Atlung & Ingmer, 1997).

In this report, we present evidence that *E. coli* cells starved for \( \text{P}_i \) under aerobic conditions maintain an active aerobic metabolism for several days, which results in dramatic changes in the composition of the culture medium. However, cell viability is not particularly affected because of the protection afforded against ROS by the expression of the RpoS and LexA regulons, and probably by the synthesis of polyamines. We also show that H-NS plays a key role in co-ordinating metabolic and protective processes in \( \text{P}_i \)-starved cells.

**METHODS**

**Bacterial strains.** These are listed in Table 1. *E. coli* strain ENZ361 is a derivative of strain AB1157 (Bachmann, 1996). It should be noted that these strains, which carry the thr-1(Alm) mutation, are still Thr~ despite the presence of the weak amber suppressor *supE44 (glnV44)* (Mount & Kosel, 1975; Eggertsson & Söll, 1988). In contrast, it appeared that the rpoS(Alm) mutation carried by strain AB1157 (Visick & Clarke, 1997) and, thus, by strain ENZ361 is partially suppressed by *supE44*, as suggested by the use of the catalase
Table 1. Escherichia coli K-12 strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENZ361</td>
<td>F' thr-1(Am) ara-14 leuB6 Δ(argF–lac)U169 tex-33 glnV44 (supE44) galK2 sulA211 rac– hisG4(Oc) rfbD1 mgl-51 rpoS396(Am) rpoL31 kgdK51 xyl-5 mit-1 argE3(Oc) thi-1</td>
<td>Dri &amp; Moreau (1993)</td>
</tr>
<tr>
<td>ENZ376</td>
<td>ΔInd− pslA::lacZ lysogen of ENZ361</td>
<td>Dri &amp; Moreau (1993)</td>
</tr>
<tr>
<td>ENZ408</td>
<td>As ENZ361 but hns-205::Tn10</td>
<td>Higgins et al. (1988)*</td>
</tr>
<tr>
<td>ENZ409</td>
<td>As ENZ376 but hns-205::Tn10</td>
<td>Dri &amp; Moreau (1993)</td>
</tr>
<tr>
<td>ENZ616</td>
<td>As ENZ361 but lexA71::Tn5(Def)</td>
<td>Krueger et al. (1983)*</td>
</tr>
<tr>
<td>ENZ618</td>
<td>As ENZ361 but Δ(srl recA)306::Tn10</td>
<td>Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ625</td>
<td>As ENZ361 but recG263::kan</td>
<td>Mandal et al. (1993)*</td>
</tr>
<tr>
<td>ENZ644</td>
<td>As ENZ361 but recO1504::Tn5 recF332::Tn3</td>
<td>Kolodner et al. (1985); Blanar et al. (1984)*</td>
</tr>
<tr>
<td>ENZ678</td>
<td>As ENZ361 but recB268::Tn10</td>
<td>Lloyd et al. (1987)*</td>
</tr>
<tr>
<td>ENZ679</td>
<td>As ENZ376 but recB268::Tn10</td>
<td>Lloyd et al. (1987)*</td>
</tr>
<tr>
<td>ENZ701</td>
<td>As ENZ361 but malB45 zja-505::Tn10 lexA3(Ind−)</td>
<td>Ossanna &amp; Mount (1989)*</td>
</tr>
<tr>
<td>ENZ702</td>
<td>As ENZ361 but rnuA60::Tn10</td>
<td>Sharples et al. (1990)*</td>
</tr>
<tr>
<td>ENZ720</td>
<td>As ENZ361 but sup-720</td>
<td>Spontaneous suppressor Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ725</td>
<td>As ENZ361 but srl-300::Tn10</td>
<td>Lange &amp; Hengge-Aronis (1991)*</td>
</tr>
<tr>
<td>ENZ766</td>
<td>As ENZ361 but rpoS359::Tn10 (null)</td>
<td>Yamada et al. (1991)*</td>
</tr>
<tr>
<td>ENZ768</td>
<td>As ENZ361 but hns::neo</td>
<td>Sharples et al. (1990); Mandal et al. (1993)*</td>
</tr>
<tr>
<td>ENZ833</td>
<td>As ENZ361 but rnuA60::Tn10 recG263::kan</td>
<td>Singer et al. (1989)*</td>
</tr>
<tr>
<td>ENZ840</td>
<td>As ENZ720 (sup-720) but nadA57::Tn10</td>
<td>Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ849</td>
<td>As ENZ720 (sup-720) but srl-300::Tn10</td>
<td>Spontaneous revertant Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ858</td>
<td>As ENZ361 but Arg*</td>
<td>Spontaneous revertant Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ862</td>
<td>As ENZ858 but Thr*</td>
<td>Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ868</td>
<td>As ENZ862 but His*</td>
<td>Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ875</td>
<td>As ENZ858 (Arg*) but srl-300::Tn10</td>
<td>Stim-Herndon et al. (1996)*</td>
</tr>
<tr>
<td>ENZ878</td>
<td>As ENZ868 (Arg* Thr* His*) but srl-300::Tn10</td>
<td>ENZ840*</td>
</tr>
<tr>
<td>ENZ885</td>
<td>As ENZ361 but adiA::MudI1734(kan)</td>
<td>Peatther et al. (1983)*</td>
</tr>
<tr>
<td>ENZ897</td>
<td>As ENZ361 but nadA57::Tn10 sup-720</td>
<td>Willis et al. (1981)*</td>
</tr>
<tr>
<td>ENZ898</td>
<td>As ENZ361 but nadA::Tn10 lys(TSuUA/G) (supG)</td>
<td>Schneider et al. (1998)*</td>
</tr>
<tr>
<td>ENZ963</td>
<td>As ENZ862 (Arg* Thr*) but srl-300::Tn10</td>
<td>Schneider et al. (1998)*</td>
</tr>
<tr>
<td>ENZ1214</td>
<td>As ENZ361 but astB::kan</td>
<td>M. Cashel, NIH, Bethesda, MD, USA</td>
</tr>
<tr>
<td>ENZ1215</td>
<td>As ENZ361 but astC::kan</td>
<td></td>
</tr>
<tr>
<td>MG1655</td>
<td>F− rph-1 rfb-50</td>
<td></td>
</tr>
</tbody>
</table>

*The source indicates the origin of the strain from which the new mutation was obtained.

assay (H₂O₂ → O₂ + H₂O) previously employed by Lange & Hengge-Aronis (1991) and Zambrano et al. (1993) to characterize rpoS mutant strains. In fact, introduction into strain ENZ361 of a null rpoS mutation reduced the level of expression of RpoS-controlled catalases (KatE and KatG), as revealed by an increase in the time required to see the production of H₂O₂, which suggests that strain ENZ361 possesses an intermediate level of RpoS activity between rpoS (null) and rpoS+ strains. Moreover, subtle but reproducible differences in catalase activity could be observed between strains carrying either rpoS(Am) supE44 mutations (e.g. strain ENZ361) or rpoS(Am) supE44 supG (sup-720) mutations (e.g. strain ENZ720), which supports the idea that these strains possess different RpoS-activity levels, at least in the experimental conditions used to perform the catalase assay (1-d-old bacteria on LB agar medium).

During strain construction, the introduction of mutations was achieved by P1 transduction (Miller, 1972). The srl-300::Tn10 mutation from strain JC10244 (Willis et al., 1981) was transduced into various strains to help distinguish bacterial populations in mixed-culture experiments. Localization of the sup-720 locus was accomplished by using conjugational and transductional methods with strains (kindly provided by B. Michel) constructed respectively by Wanner (1986) and Singer et al. (1989), which carry the transposon Tn10 at defined positions in the E. coli genome. Backmutants (e.g. Arg* revertants) were isolated as fast-growing revertants (suppressors often decrease the growth rate) (Eggertsson &
Soll, 1988), scored for lack of reversion of nonselected mutations, and tested for their catalase activity to ensure that they behaved as the ENZ361 parental strain.

### Media and growth conditions

The minimal medium used for liquid cultures was essentially the MOPS medium described by Neidhardt et al. (1974) containing 86 mM NaCl, 9.5 mM NH₄Cl, 5 mM K₂HPO₄, and 20.2 mM glucose (0.04%, w/v) supplemented with five vitamins (0.02 mM thiamine, 0.02 mM calcium pantothenate, 0.02 mM p-aminobenzoic acid, 0.02 mM P-hydroxybenzoic acid and 0.02 mM 2,3-dihydroxybenzoic acid; Neidhardt et al., 1977) and six amino acids (0.04 mM threonine, 0.8 mM leucine, 0.2 mM histidine, 0.4 mM arginine, 0.4 mM isoleucine and 0.6 mM valine; Neidhardt et al., 1977); the pH was 7.2. In Pₗ-limiting medium, the concentration of K₂HPO₄ was reduced from 0.4 to 0.04 or 0.05 mM. After aeration, the concentration of potassium as in MOPS medium (Dri, 1979) was supplemented with 1 mM MgSO₄, 0.1 mM CaCl₂, 0.03 mM thiamine, 0.2% (w/v) glucose, amino acids when required (0.04 mM threonine, 0.8 mM leucine, 0.2 mM histidine, 0.4 mM arginine, 0.4 mM isoleucine, 0.6 mM valine; Neidhardt et al., 1977) and 0.4 mM arginine, 0.4 mM isoleucine and 0.6 mM valine (Neidhardt et al., 1977). For the liquid cultures, the medium was essentially the MOPS medium described by Neidhardt et al. (1974) for 24 h, collected by centrifugation, resuspended in the same volume of MOPS buffer, and serial dilutions were spread in triplicate on LB and LB-tetracycline agar media to determine the total number of viable cells and the number of revertants. Tetracycline (Tc) was used at 12 μg ml⁻¹. All incubations were at 37°C.

### Long-term starvation experiments

Bacteria were grown in MOPS medium for 24 h, collected by centrifugation, resuspended in the same volume of MOPS buffer, diluted 1:200 into 50 ml MOPS medium limited in P, or in glucose (0.04%, w/v) in 500 ml Erlenmeyer flasks (time zero), and further incubated with aeration in a shaking water bath. Every 4 or 5 d, water was added to cultures to compensate for evaporation. At specified time intervals, 0.5 ml samples were withdrawn, cells were collected by centrifugation, resuspended in M9 buffer, and serial dilutions were spread in duplicate with 3 ml soft agar on M9 agar medium containing appropriate concentrations of amino acids in order to measure titres of viable cells and revertants. C.f.u. were counted after 3 d (total cells) or 6 d (revertants) of incubation. Each graph represents data obtained from one experiment and is representative of several experiments. Variations between experiments in the concentrations of revertants were less than 10% at each time point.

### Mixed-culture experiments

Of the two strains used, one (generally that used as the minor population) carried a Tn10 (Tc⁰) antibiotic-resistance marker. Unless otherwise indicated (Fig. 3d), Tc⁰ and Tc⁰ bacteria were grown for 24 h in MOPS medium limited in glucose (0.04%, w/v), diluted 1:200 into MOPS medium limited in P, or glucose (time zero), grown for 24 h, and mixed (day 1) by transferring 50 μl of a 1:100 dilution in MOPS of the culture used as the minority into 50 ml of a culture used as the majority (Zambrano et al., 1993). The mixed culture was further incubated with agitation; aliquots were removed at appropriate intervals and centrifuged, and bacteria were resuspended, serially diluted in M9, and plated in triplicate onto LB and LB-tetracycline agar media to determine the total number of viable cells and the number of Tc⁰ bacteria, respectively. In the case of nonsense suppressors, variations between experiments in the concentrations of revertants were less than one log at each time point, and data are averaged. In contrast, in the case of Arg⁰, His⁰ and/or Thr⁰ revertants, more variations were observed and data from several independent experiments are shown.

### Growth in spent culture medium

Cultures were grown in Pₗ-limiting medium; 8 ml aliquots were taken at appropriate intervals, and the cells were removed by centrifugation and filtration. The spent culture media were distributed into 16 mm glass test tubes (1 ml aliquots), and supplemented with nutrients (40–50 μl). ENZ361 bacteria grown overnight in MOPS medium limited in glucose (0.05%, w/v) were centrifuged, resuspended in the same volume of MOPS, and used to inoculate (10 μl) the supplemented spent culture media. Cultures were incubated for 24 h with gentle agitation, and aliquots were serially diluted and plated in triplicate on LB agar medium to measure the number of viable cells. The experiment-to-experiment variation in the number of viable cells was less than 30% at each time point.

### Determination of glucose and acetate concentrations

The determination of the concentrations of glucose and acetate in culture media was based on the enzymic formation of NADH measured by the increase in absorbance at 340 nm. Glucose concentration was determined by the coupled enzyme reaction catalysed by hexokinase and glucose-6-phosphate dehydrogenase (glucose HK assay from Sigma); analysis of acetate used acetyl-CoA synthetase, citrate synthase and malate dehydrogenase (acetate assay from Boehringer). Assays were performed in duplicate according to the manufacturers’ instructions. Variations between experiments were less than 10%.

### Assay of β-galactosidase

To measure the expression of the sulA::lacZ fusion (Huisman & D’Ari, 1983), 1 ml samples were withdrawn and brought to a final concentration of 200 μg chloramphenicol ml⁻¹ to stop protein synthesis. β-Galactosidase measurements were carried out in duplicate as described previously (Dri & Moreau, 1994). Activities are expressed per OD₆₅₀ unit of cell suspensions measured with a Jasco V530 spectrophotometer in cells of 1 cm path length by using the following formula (after Miller, 1972):

\[
[\text{OD}_{650}] = \left[1.75 \text{OD}_{650} \times \text{reaction volume} \times 1000\right] / (\text{reaction time} \times \text{sample volume} \times \text{OD}_{650})
\]

OD₆₅₀ and OD₆₅₀ were read from the reaction mixture and time was in minutes.

### UV irradiation

UV light of predominantly 254 nm was obtained from a 15 W germicidal lamp. The lamp output was about 1.5 J m⁻² s⁻¹. Samples in MOPS medium (1.8 ml in a 50 mm glass Petri dish) were irradiated for 5 s at 4°C, and 1.5 ml portions in 16 mm glass test tubes were further incubated with agitation for 1 h at 37°C.

### RESULTS

#### Degradation of exogenous arginine, threonine and glucose by Pₗ-starved cells

The possibility that E. coli cells starved for Pₗ under aerobic conditions may suffer from high cellular levels of ROS prompted us to determine whether this could increase the mutation rate in strain ENZ361, which carries notably the thr-1(Am), hisG+4(Oc) and argE3(Oc) mutations. At first sight, this seemed to be the case because, if the frequency of rifampicin-resistant mutants did not change significantly in cultures starved for Pₗ or glucose (R. Duval & P. L. Moreau, unpublished results),
were grown to saturation in MOPS medium containing a limited amount of histidine; identical results were obtained when the medium contained no histidine. Survival (a) was determined as the viable cell concentration at each point divided by the viable cell concentration on day 1 (6.2 \times 10^8 and 5.5 \times 10^8 ml^{-1} in P_i^- and in G^- medium, respectively). The frequency of His\(^+\) revertants (b) was determined as the concentration of revertants divided by the concentration of viable cells at each point. Data are from one representative experiment. Similar time courses and yields of revertants were obtained whether the selection was for the His\(^+\), Arg\(^+\) or Thr\(^+\) phenotype.

Fig. 1. Accumulation of prototrophic revertants during prolonged incubation in P\(_i\)-limiting medium. E. coli ENZ361 cells were grown to saturation in MOPS medium (2.5 \times 10^5 cells ml\(^{-1}\)), centrifuged, resuspended in the same volume of MOPS buffer, diluted 1:200 (designated time zero) into fresh medium limited in P\(_i\) (P\(_i\)^- (•)) or in glucose (G^- (○)), and incubated further with aeration. Samples were withdrawn at the times indicated from day 1 through day 10 for measurements of the concentrations of viable cells (determined on M9 minimal agar medium) and His\(^+\) revertants (selected on M9 agar medium containing a limited amount of histidine; identical results were obtained when the medium contained no histidine). Survival (a) was determined as the viable cell concentration at each point divided by the viable cell concentration on day 1 (6.2 \times 10^8 and 5.5 \times 10^8 ml^{-1} in P_i^- and in G^- medium, respectively). The frequency of His\(^+\) revertants (b) was determined as the concentration of revertants divided by the concentration of viable cells at each point. Data are from one representative experiment. Similar time courses and yields of revertants were obtained whether the selection was for the His\(^+\), Arg\(^+\) or Thr\(^+\) phenotype.

the frequency of prototrophic revertants did increase dramatically in cultures starved for P\(_i\), but not in cultures starved for glucose (Fig. 1). By using Hfr-mating and P1-transduction techniques, we determined that a typical Thr\(^+\) His\(^+\) Arg\(^+\) revertant isolated from a 20-d-old culture in P\(_i\)-limiting medium (strain ENZ720) carried a suppressor mutation, tentatively designated as sup-720, that was co-transducible at 92% with the nadA::TnlO marker; sup-720 would be therefore equivalent to the known supG (supL) suppressor that is located close to nadA. The supG suppressor results from a single base substitution in lysT that changes the anticodon of the lysine tRNA in such a way that both ochre and amber nonsense codons can be recognized (SuUUA/G) (Prather et al., 1983; Eggertsson & Söll, 1988).

Mixed-culture experiments clearly indicate, however, that the suppressors produced in cultures starved for P\(_i\) result from the growth of rare spontaneous mutants, rather than from an increase in the mutation rate in P\(_i\)-starved cells. When sup-720 suppressors carrying a srl::TnlO (Tc\(^+\)) marker (strain ENZ849), grown for 1 d in P\(_i\)-limiting medium (or in glucose-limiting medium), were added as a minor population into a 1-d-old culture of ENZ361 cells grown in P\(_i\)-limiting medium, the former Tc\(^+\) suppressors started to grow after 2 further days of incubation, and reached a maximal concentration of about 10^6 cells ml\(^{-1}\) after 3 more days of incubation, while the total number of viable cells was about 4 \times 10^8 cells ml\(^{-1}\) (Fig. 2 and data not shown), which mimics the normal production of revertants in a culture of ENZ361 cells starved for P\(_i\) (Fig. 1). In contrast, sup-720 suppressors grown in P\(_i\)-limiting or in glucose-limiting medium could not grow and eventually died when they were added as a minor population into a culture starved for glucose (data not shown).

Transduction into strain ENZ361 of the sup-720 mutation (yielding strain ENZ897) or of a true supG mutation (yielding strain ENZ898) gave rise to strains that grew in mixed cultures like sup-720 mutants isolated from an old culture (strain ENZ849) (Fig. 2), which suggests that the presence of a single supG mutation is sufficient to permit cells to grow in a culture of ENZ361 bacteria starved for P\(_i\). We can rule out the possibility that the presence of the TnlO (Tc\(^+\)) antibiotic-resistance marker used to distinguish the populations in mixed-culture experiments could affect the cells' growth because identical results were obtained whether this marker was introduced into strains placed either as the

Fig. 2. Growth of supG suppressors in mixed culture. Strains ENZ725 (srl::TnlO (●)), ENZ849 (sup-720 srl::TnlO (□), ENZ897 (nadA::TnlO sup-720) (▼) and ENZ898 (nadA::TnlO supG) (▲) were grown for 1 d in P\(_i\)-limiting medium, and rediluted 1:10\(^5\) into 1-d-old cultures of strain ENZ361 (Tc\(^+\)) in P\(_i\)-limiting medium. Cell mixtures were further incubated for the times indicated, and assayed for viable cells on LB-tetracycline. The total number of viable cells measured on LB medium decreased from about 6 \times 10^6 ml^{-1} on day 1 to about 4 \times 10^6 cells ml\(^{-1}\) on day 8 of incubation. Data are the means of two independent experiments.
minor or as the major population in mixed cultures (data not shown), and introduction of the srl::Tn10 mutation into strain ENZ361 (yielding strain ENZ725) did not permit cells to grow in mixed culture (Fig. 2).

To determine whether reversion of the thr-1(Am), hisG4(Oc) and/or argE3(Oc) mutations in strain ENZ361 could account, at least in part, for the growth of supG suppressors in cultures starved for P_i, spontaneous revertants of strain ENZ361 (Thr', His+ or Arg') were isolated on M9 minimal medium (see Methods), and tested for their ability to grow in mixed culture. Fig. 3(a) shows that Arg' cells (strain ENZ875) could grow in mixed culture like supG suppressors, whereas Thr' or His' cells could not grow (data not shown). We checked that the Arg' cells had not accumulated secondary mutations suppressing the thr or his mutations during growth in mixed culture (data not shown). Moreover, Fig. 3(a, b, c) shows that the strains selected successively as Arg', Thr' (Arg+), and His' (Arg' Thr') all behaved similarly in mixed cultures, which supports the idea that supG suppressors could grow in a culture of ENZ361 cells starved for P_i primarily because of the reversion of the argE3(Oc) mutation.

However, when Arg' bacteria [i.e. Arg' revertants (Fig. 3a, b, c), supG suppressors (ENZ898) (Fig. 2), or MG1655 wild-type bacteria (data not shown)] were added as a minor population into 1-d-old cultures of ENZ361 cells starved for P_i, the former bacteria started to grow only after a delay of several days. This delay could reflect either the period of time necessary for Arg' bacteria to somehow adapt themselves to the growth conditions, or the period of time necessary for ENZ361 cells to condition the culture medium so as to permit Arg' bacteria to grow. The latter hypothesis appeared to be correct because Arg' revertants, as well as Arg' Thr' and Arg' Thr' His' revertants, could start growing immediately without a delay when they were added into 3-d-old cultures (Fig. 3d).

To help define changes that occur in the composition of the P_i-limiting medium during incubation of ENZ361 cells, we determined which nutrients needed to be added into sterilized spent culture media taken at different time intervals to allow fresh ENZ361 cells, added at a low cell density, to grow to saturation (about 10^9 cells ml^-1) (Fig. 4a). As expected, by 7 h of incubation, when the growth rate of ENZ361 cells in P_i-limiting medium decreased abruptly (cells entered stationary phase), P_i was the only

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**Fig. 3. Growth of Arg' revertants in mixed culture.** In (a), (b) and (c), mixed-culture experiments were done with ENZ875 (Arg' srl::Tn10) (a), ENZ963 (Arg' Thr' srl::Tn10) (b) and ENZ878 (His' Arg' Thr' srl::Tn10) (c) revertants grown for 1 d in P_i-limiting medium and then rediluted 1:10^5 into 1-d-old cultures in P_i-limiting medium of strain ENZ361 (Tc') containing about 6.2 x 10^8 cells ml^-1. In (d), 1-d-old Arg' Tc' (■, □), Arg' Thr' Tc' (▲, △) and Arg' Thr' His' Tc' (○, o) revertants were rediluted 1:10^5 into 3-d-old cultures in P_i-limiting medium of strain ENZ361 (Tc') containing about 4.1 x 10^8 cells ml^-1. By day 7, the total number of viable cells in cultures was about 3.6 x 10^8 cells ml^-1. Data of several independent experiments are shown.
Fig. 4. Concentrations of nutrients in spent culture media. ENZ361 cells grown overnight in glucose-limiting medium were diluted 1:200 in P₁-limiting medium (time zero) and grown further with aeration. (a) At the times indicated, samples were withdrawn, sterilized by filtration and inoculated at a final concentration of about 6.5 x 10⁶ cells ml⁻¹ with ENZ361 cells starved for glucose. Media were either not supplemented (■), or supplemented with five nutrients (10 mM NH₄Cl, 5 mM K₂HPO₄, 10 mM glucose, 0.4 mM threonine and 0.4 mM arginine) (●), or with only four nutrients, one nutrient being omitted: NH₄Cl (□), glucose (△), P₁ (○), threonine (▽) or arginine (○). The reconstituted cultures were incubated for 24 h, and the concentrations of viable cells were determined on LB agar medium. Data are the means of two independent experiments. (b) At the times indicated, the concentrations of glucose (△) and acetate (▽) in spent culture media were determined by enzymic methods. Data are the means of four independent experiments.

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limiting factor in the culture medium. However, by day 1 (24 h), day 2 and day 3 of incubation, threonine, arginine and glucose were in turn also in limiting amounts (data not shown). When a spent culture medium taken at a given time was supplemented with fresh ENZ361 cells and all the nutrients possibly in limiting amounts but one, bacterial growth would be therefore proportional to the actual concentration in the spent culture medium of the nutrient that was omitted. The results of such an assay performed at various time intervals, and by omitting various nutrients (Fig. 4a) indicate that the same nutrients that were depleted from the culture medium (or metabolites) could be subsequently excreted to, and somewhat accumulated in, the culture medium depending on the rate at which they were reutilized. Notably, the concentration of P₁ in the culture medium appeared to increase steadily during the first 5 d of incubation (Fig. 4a; curve —P₁), which indicates that P₁ was actually excreted by non-growing bacteria. It is known that cells entering stationary phase under P₁-starvation conditions maintain high intracellular P₁ levels (about 7 mM) (Rao et al., 1993; N. W. Lutz & P. L. Moreau, unpublished results), which provides a potential source of P₁. Like P₁, threonine was totally depleted from the culture medium after 1 d of incubation, and was subsequently excreted (Fig. 4a; curve —Thr), which suggests that this amino acid was degraded only partially by P₁-starved cells. At first glance, it seemed that bacteria starved for P₁ also metabolized glucose only partially (Fig. 4a; curve —Glc). In fact, enzymic methods of analysis (Fig. 4b) showed that the concentration of glucose decreased from about 20 mM in fresh medium to undetectable levels (less than 0.05 mM) in a 3-d-old culture medium, while the concentration of acetate increased up to about 10 mM by day 2 of incubation, which indicates that P₁-starved cells can totally degrade glucose to products such as acetate. Likewise, arginine appeared to be used slowly but extensively by P₁-starved cells, as indicated by the fact that by day 2 of incubation, spent culture media supplemented with all the required nutrients except arginine could not support any bacterial growth (Fig. 4a; curve —Arg). Arginine degradation can occur through three different pathways: the arginine succinyltransferase pathway (astBC-dependent) induced by nitrogen and carbon limitation, to produce ammonia; the biodegradative arginine decarboxylase pathway (adiA-dependent) induced by anaerobic and acidic growth conditions; and the constitutive biosynthetic arginine decarboxylase pathway (speAB-dependent), used to produce putrescine (Glansdorff, 1996; Reitzer, 1996; Stim-Herndon et al., 1996; Schneider et al., 1998). We can rule out the possibility that arginine was used as a source of nitrogen because ammonium, the preferred nitrogen source, was not in limiting amount in spent culture media (Fig. 4a; curve —NH₄¹), and inactivation of astB or astC had no effect on the rate of degradation of arginine during P₁-starvation (data not shown). Likewise, inactivation of adiA had no effect on arginine degradation (data not shown). In fact, the degradation of arginine appeared to occur through the biosynthetic arginine decarboxylase pathway by SpeA activity (Glansdorff, 1996), preferentially delayed the degradation of arginine when it was added into P₁-limiting medium to a final concentration of 10 mM (data not shown).

Taken together, these results show that ENZ361 cells starved for P₁ can maintain an active metabolism for at least 3 d, which modifies the composition of the culture medium and, ultimately, the composition of the bacterial population because, when arginine is totally depleted from the culture medium, only spontaneous Arg⁻ revertants present in the culture can grow by using the nutrients, notably P₁, acetate and threonine, that are progressively excreted by the bulk of the population.
Protective role of the RpoS regulon during Pi starvation

Because supG suppressors rather than Arg⁺ revertants were found in old cultures starved for Pi (Fig. 1 and data not shown), it is probable that reversion of other nonsense mutation(s) than the argE3(Oc) mutation may provide a growth advantage to supG suppressors. Mere reversion of the thr-1(Am) mutation, or of the hisG4(Oc) mutation, or of both cannot account for this result because Arg⁺ Thr⁺ and Arg⁺ Thr⁺ His⁺ revertants did not exhibit a significant growth advantage over Arg⁺ revertants in mixed cultures (Fig. 3). Two lines of evidence then pointed to the rpoS gene. First, it has been shown recently that E. coli strains derived from AB1157, such as ENZ361, carry an amber mutation in rpoS that can be only partially suppressed by the weak amber suppressor supE44 also carried by these strains (Visick & Clarke, 1997); this seemed to be true as judged from the different catalase activities exhibited by strain ENZ361 and rpoS derivatives (see Methods). Second, Pi starvation strongly induces the expression of the rpoS gene (Lange & Hengge-Aronis, 1991), and Pi⁺-starved cells contain high cellular levels of RpoS (Gentry et al., 1993; Hengge-Aronis, 1996), which suggests that an increase in RpoS activity may somehow protect cells starved for Pi.

To assess the possible role of RpoS during Pi starvation, we constructed and studied the behaviour of an rpoS null mutant. Fig. 5 shows that introduction of the rpoS359 allele in strain ENZ361 (giving rise to strain ENZ766) provoked a dramatic decrease in cell viability between day 2 and day 4 of incubation under Pi-starvation conditions (top panels), whereas the rates of degradation of arginine and glucose were only slightly affected (lower panels). Therefore, a simple interpretation of the observation that supG suppressors rather than Arg⁺ revertants were found in Pi⁺-starved cultures is that a supG mutation would suppress the rpoS(Am) mutation more strongly than does the original supE44 mutation carried by strain ENZ361, thereby increasing RpoS activity, cell viability under Pi-starvation conditions, and hence ability to grow in old cultures. This idea was further supported by the finding that strain MG1655 (rpoS⁺) exhibits a viability slightly higher than strain ENZ361 [rpoS(Am) supE44] under Pi⁺-starvation conditions (see Fig. 7a, b).

Recombinational repair of DSB in Pi⁺-starved cells

The possibility that a better protection of Arg⁺ revertants, through the expression of RpoS-controlled genes, could increase their ability to grow in a culture starved for Pi, prompted us to test the role of LexA-controlled DNA repair genes (Walker, 1996), which are also induced under Pi⁺-starvation conditions (Dri & Moreau, 1993). Mutations that prevent induction of the LexA regulon (lexA-Ind⁻; Fig. 6a) or that inhibit recombinational repair, i.e. recA (Fig. 6b) and ruvA (Fig. 6c), delayed the production of revertants in a culture starved for Pi⁺, whereas inactivation of other DNA repair genes, such as wrA, whose product catalyses excision of
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**Fig. 6.** Altered ability of recombination mutants to accumulate prototrophic revertants during prolonged incubation in Pi-limiting medium. Experiments were done as described in the legend to Fig. 1. For each strain, the total number of viable cells measured on day 1 of incubation is indicated in parentheses: (a) ENZ616 (lexA-Def) \((4.7 \times 10^8 \text{ cells ml}^{-1})\) (■) and ENZ701 (lexA-Ind) \((5.6 \times 10^8 \text{ cells ml}^{-1})\) (○); (b) ENZ618 (recA) \((3.6 \times 10^8 \text{ cells ml}^{-1})\) (●) and ENZ644 (recFO) \((4.1 \times 10^8 \text{ cells ml}^{-1})\) (□); (c) ENZ625 (recG) \((4.7 \times 10^8 \text{ cells ml}^{-1})\) (□), ENZ702 (ruvA) \((2.5 \times 10^8 \text{ cells ml}^{-1})\) (●) and ENZ833 (ruvA recG) \((8.5 \times 10^7 \text{ cells ml}^{-1})\) (●); and (d) ENZ678 (recB) \((2.2 \times 10^8 \text{ cells ml}^{-1})\). Values obtained with parental strain ENZ361 \((6.6 \times 10^8 \text{ cells ml}^{-1})\) (□) are reported for comparison. For each strain, the data are from a representative experiment from several separate trials.

**Table 2.** The recB mutation prevents the induction of the sulA::lacZ fusion in P1-starved cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>β-Galactosidase activity</th>
<th>Exponential growth</th>
<th>P1 starvation 28 h</th>
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<tr>
<td></td>
<td>Untreated + UV + Nal</td>
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<tr>
<td>ENZ376</td>
<td>137 ± 19 2840 ± 457 3360 ± 385 1328 ± 31</td>
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<td></td>
</tr>
<tr>
<td>ENZ409 (hns-205)</td>
<td>212 ± 32 2207 ± 345 2322 ± 386 501 ± 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENZ679 (recB)</td>
<td>30 ± 5 842 ± 278 36 ± 2 49 ± 2</td>
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<td></td>
</tr>
</tbody>
</table>

bulky lesions, had no effect (data not shown), which suggests that recombinational repair may protect P1-starved cells. The investigation was thus extended to other recombination genes that are not under the control of LexA. Although introduction of a recG mutation, which disables a helicase required with RuvABC to process RecA-mediated recombination intermediates (i.e. Holliday junctions) (Lloyd & Low, 1996), had only a slight inhibiting effect on the rate of production of revertants (Fig. 6c), inactivation of both ruvA and recG genes prevented any accumulation of revertants (Fig. 6c), which confirms the involvement of recombination mechanisms under P1-starvation conditions. Alterations in the recF, recO and recR genes (Fig. 6b and data not shown), whose products are collectively required to initiate recombinational repair of single-strand gaps generated from bulky lesions (Umezu & Kolodner, 1994; Cox, 1997) had, however, no effects on the production of revertants, which excludes the possibility that such DNA damage may occur in significant amount in P1-starved cells. In contrast, the production of revertants was significantly affected by introduction of a recB mutation (Fig. 6d) that affects RecBCD, an enzyme required to initiate recombinational repair of DSB.
Fig. 7. Effects of ruvA recG and hns mutations on viability and metabolism under Pi-starvation conditions. Experiments were done as described in the legend to Fig. 5 with strains (a) MG1655 (wild-type), (b) ENZ361, (c) ENZ383 (ENZ361 ruvA recG), (d) ENZ408 (ENZ361 hns-205::Tn10) and (e) ENZ768 (ENZ361 hns::neo). Spent media were either not supplemented (●), or supplemented with four nutrients (5 mM K$_2$HPO$_4$, 10 mM glucose, 0.4 mM threonine and 0.4 mM arginine) (○), or with only three nutrients, one nutrient being omitted: glucose (▲), P$_i$ (○), threonine (▽) or arginine (○). Concentrations of acetate (▼) and glucose (▲) are shown in the bottom panels. Data in all panels are the means of two independent experiments.
RecBCD binds exclusively to double-stranded DNA with nearly flush ends, and generates through its helicase and nuclease activities single-stranded DNA (ssDNA) used eventually by RecA to promote recombination and repair (Kowalczykowski et al., 1994). Taken together, these data imply (i) that DSB are produced during the first days of incubation following starvation for Pi, (ii) that these potentially lethal lesions are repaired by the combined activities of the RecBCD, RecA, RecG and RuvABC recombination proteins, and (iii) that the efficiency of repair is enhanced through the induction of the LexA regulon which includes recA and ruvAB.

**Induction of the LexA regulon is triggered by DSB produced under aerobic growth conditions**

Induction of LexA-controlled genes is triggered by the same ssDNA that is eventually implicated in RecA-mediated recombination; this is because the RecA filament formed with ssDNA first stimulates the cleavage of the LexA repressor before promoting strand exchange (Moreau, 1987). In the case of DSB generated by radicals or nalidixic acid (an inhibitor of DNA gyrase), cleavage of the LexA repressor and induction of LexA-controlled genes such as recA or sulA thus require the activity of the RecBCD enzyme to generate ssDNA and facilitate the loading of RecA (Moreau, 1988; Sassanfar & Roberts, 1990; Anderson & Kowalczykowski, 1998). As shown in Table 2, induction of the LexA regulon, measured by using a sulA::lacZ fusion, was dramatically reduced following starvation for Pi, as well as treatment with nalidixic acid in strain ENZ679, which carries a recB mutation; this indicates that DSB are the main DNA damage that triggers induction of the LexA regulon in P_i-starved cells.

The level of expression of the sulA::lacZ fusion was also dramatically reduced in exponentially growing recB mutants (strain ENZ679) compared to recB+ cells (strain ENZ376) (Table 2), which is in good agreement with the notion that DSB are normally produced in growing cells (Lloyd & Low, 1996) due to the production of H_2O_2 by aerobic metabolism (González-Flecha & Depp, 1997). Likewise, the level of expression of the sulA::lacZ fusion in strain ENZ376 appeared to be strictly dependent upon the level of aeration of the cultures starved for P_i; the higher the level of aeration, the higher the level of expression of the sulA promoter (P.L. Moreau, unpublished results), which suggests that DSB produced in P_i-starved cells also result from aerobic metabolism.

**H-NS helps maintain a rapid degradation of arginine and glucose**

Because H-NS is required for a full induction of the LexA regulon in P_i-starved cells (Dry & Moreau, 1993; Table 2), the potential role of H-NS during P_i starvation was further examined. Surprisingly, it appeared that cultures of hns mutant bacteria starved for P_i could neither accumulate prototrophic revertants upon prolonged incubation, nor permit hns+ supG revertants to grow in mixed cultures (data not shown). These results can be simply explained, however, by the finding that hns mutant bacteria starved for P_i exhibited much reduced rates of arginine degradation compared to hns+ cells (Fig. 7d, e): an excess of arginine in the growth medium is indeed expected to prevent Arg+ revertants from expressing any growth advantage over argE parent bacteria. The inhibiting effect of hns mutations was not limited to arginine degradation since the rate of glucose degradation was also reduced about twofold under P_i-starvation conditions in hns mutants compared to hns+ cells (Fig. 7d, e). As expected, the inhibition of arginine and glucose degradation was even stronger in hns::neo mutants (Fig. 7e), which produce practically no H-NS protein, than in hns-205::Tn10 mutants (Fig. 7d), which produce a truncated H-NS protein with some residual activity (Dersch et al., 1994). Although these results are reminiscent of those observed with ruvA recG mutant bacteria (Fig. 6c, 7c), the viability of hns mutants, in contrast to that of ruvA recG mutants, was not specifically affected during prolonged incubation under P_i-starvation conditions (Fig. 7c, d, e), which suggests that a primary role of H-NS in P_i-starved cells is to sustain metabolic activities rather than DNA repair mechanisms (and indirectly cell metabolism). This notion may, however, help explain the inhibiting effect of hns mutations on the expression of the LexA regulon under P_i starvation conditions because a lower rate of metabolism in P_i-starved hns mutants may decrease the rate of production of ROS, the amount of DSB, and thus the level of induction of LexA-controlled DNA-repair genes.

**DISCUSSION**

We show here that E. coli strains ENZ361 and MG1655 (wild-type) incubated under aerobic, P_i-starvation conditions still exhibit a high viability, although they totally degrade exogenous glucose to products such as acetate. Does this mean that P_i-starved cells, like apparently glucose-starved cells (Nyström et al., 1996), use an-aerobic (Pfl) or semi-aerobic (PoxB) enzymes to metabolize glucose to acetate, which decreases the activity of the aerobic respiratory chain and, thus, the generation of potentially lethal ROS? This is probably not the case, for the following reasons. First, the fermentative enzyme pyruvate formate-lyase (Pfl), which is thought to be used by glucose-starved cells to produce acetate (Nyström, 1994; Nyström et al., 1996), is synthesized in dramatically reduced amounts in P_i-starved cells (VanBogelen et al., 1996). Second, pyruvate oxidase (PoxB), which may be preferentially used to produce acetate when bacteria approach the stationary phase, is unlikely to play a key role under P_i-starvation conditions because the expression of poxB is strictly controlled by RpoS (Chang et al., 1994), and the metabolism of glucose to acetate was essentially independent of RpoS in P_i-starved cells. Therefore, cells starved for P_i should mainly use the PDH (NAD-dependent)/Pta/AckA shunt to metabolize pyruvate to acetate (Fig. 8), just as cells growing under aerobic,

If the above conclusion is correct, P1-starved cells might be exposed to high levels of ROS generated by enzymes of the respiratory chain while glucose is metabolized. Several lines of evidence show that this is the case. As indicated by the growth kinetics of nonsense suppressors in cultures starved for P1, survival and metabolism of starved cells was dependent upon RecBCD, RecA, RecG and RuvABC, which are required to repair DSB (Lloyd & Low, 1996), a damage produced mainly through hydroxyl radical attack on DNA sugar (Keyer & Imlay, 1996; Henle & Linn, 1997). As expected, since repair of DSB through recombinational processes is essentially error-free (Lloyd & Low, 1996), the mutation rate was not significantly increased in P1-starved cells. The notion that P1-starved cells are exposed to ROS attack, and that repair of DNA damage (i.e. DSB) requires recombination proteins helps explain (i) the requirement in these cells of the induction of the LexA regulon (lexA+ genotype), and (ii) the fact that the LexA regulon is actually induced in P1-starved cells (Dri & Moreau, 1993) as in cells exposed to increased superoxide production (Brawn & Fridovich, 1985), which may enhance the DNA-repair efficiency by increasing the cellular levels of RecA and RuvAB proteins. That the efficiency of induction of the LexA regulon in P1-starved cells depends upon the level of aeration of the cultures supports the idea that DNA is then damaged because of aerobic metabolism. Finally, the finding that induction of the LexA regulon in P1-starved cells is totally prevented by introduction of a recB mutation clearly indicates that DSB are the main DNA damage produced during starvation for P1.

The requirement of RpoS-dependent functions for survival of P1-starved cells while glucose was metabolized may thus be explained by the key role played by RpoS-controlled gene products (i.e. KatG, KatE and Dps) in the defence of DNA against ROS (Hengge-Aronis, 1996). This idea is supported by the finding that the level of induction of the LexA regulon in P1-starved cells is inversely related to RpoS activity, which suggests that the higher the RpoS activity, the lower the number of DSB, and thus the lower the level of...
induction of the LexA regulon (P. L. Moreau, unpublished results). Likewise, the degradation of threonine and arginine observed under Pi-starvation conditions may be explained by the synthesis of putrescine and spermidine (Fig. 8), two polyamines that help protect nucleic acids by scavenging ROS (Chol Ha et al., 1998).

The protective role of polyamines against ROS, and the observation that ribosomes bind virtually all the intracellular spermidine (Davis et al., 1992), might also help explain the results obtained by Davis et al. (1986), who found that E. coli cells extensively degrade their ribosomes and die at the onset of P_i starvation. Indeed, the strain (D10) used by these authors was Met^- and, hence, might produce reduced amounts of spermidine. This idea is supported by the fact that many spontaneous metK mutations cause methionine auxotrophy and dramatically reduce S-adenosylmethionine synthetase activity (Sathishchandran et al., 1990); a defect in metK expression and, hence, in spermidine synthesis might account for the exceptional sensitivity of strain D10 to P_i starvation when the aggregation to ribosomes by ROS is high. Taken together, these data support the idea that E. coli can normally survive starvation for P_i despite the maintenance of an aerobic metabolism, because of efficient protections against ROS provided notably by the synthesis of polyamines, and by the enhanced expression of the RpoS and LexA regulons.

Besides RpoS and LexA, we show that H-NS also plays a key role in P_i-starved cells. First of all, it should be noted that the intracellular concentration of a predominant isoform of H-NS is increased about fourfold at the onset of P_i starvation (VanBogelen et al., 1996) while DNA concentration is increased by about 20% (P. L. Moreau, unpublished results), which suggests that the ratio of H-NS to DNA and, thus, the activity of H-NS may be significantly increased in P_i-starved cells. Our results indicate that a role of H-NS is to maintain a rapid metabolism of glucose to acetate in P_i-starved cells. How could H-NS affect such a metabolism? Recent data indicate that in growing bacteria H-NS is required for the synthesis of glycolytic enzymes including the AceF and Lpd subunits of the aerobic PDH complex encoded by the aceEF/lpd operon (Smith & Neidhardt, 1983; Quail et al., 1994; Laurent-Winter et al., 1997). A simple interpretation of our results is therefore that H-NS may also stimulate, directly or indirectly, the synthesis of these enzymes in P_i-starved cells, thereby increasing the activity of the aerobic PDH complex. Moreover, our results indicate that H-NS is required for the degradation of arginine by the arginine decarboxylase SpeA-initiated pathway, which suggests that H-NS may somehow enhance SpeA activity. Interestingly, it has been shown that H-NS inhibits the expression of the other arginine decarboxylase, encoded by adiA, that is expressed under anaerobic conditions (Shi et al., 1993), which further supports the idea that H-NS may generally favour aerobic versus anaerobic catabolic processes in P_i-starved cells.

In contrast to the complete degradation of arginine and glucose that takes place in hns^- cells starved for P_i, the degradation of threonine occurred inefficiently, and threonine could be excreted to the culture medium. This surprising result is, however, in good agreement with the fact that, in growing bacteria, H-NS inhibits the expression of the kbl/tdh operon encoding enzymes that initiate threonine degradation (Landgraf et al., 1994; Reitzer, 1996). In P_i-starved cells, a possible role for this H-NS inhibitory effect may be to trigger the synthesis of homoserine lactone, an activator of RpoS that is thought to be produced through the feedback inhibition by threonine of enzymes in the threonine biosynthetic pathway (Huisman & Kolter, 1994) (Fig. 8). In the same view, it should be noted that the degradation of glucose to acetate may also enhance the activity of RpoS in two different ways, through a decrease in the intracellular concentration of UDP-glucose, an inhibitor of RpoS activity (Böhringer et al., 1995), and through an increase in the intracellular concentration of acetate, an activator of the RNA polymerase-RpoS (Eo^r) holoenzyme (Ding et al., 1995). Therefore, in P_i-starved cells, a role of H-NS may be to increase the activity of RpoS, whereas H-NS somehow decreases the cellular concentration of RpoS in exponentially growing cells, an inhibiting effect that disappears, however, when cells enter stationary phase (Barth et al., 1995). In sum, the present work suggests that H-NS plays two complementary roles in P_i-starved cells: it helps maintain an aerobic metabolism and, thus, a high level of energy at least while glucose is in excess, and it increases the cellular defences against ROS that are then produced by increasing RpoS activity.

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