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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 (P_i)-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 P_i 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 P_i-starved cells. It is shown that induction of the LexA regulon, which helps protect P_i-starved cells, is totally prevented by introduction of a *recB* mutation, which indicates that DSB are actually the main DNA lesion generated in P_i-starved cells. The requirement of RpoS for survival of cells starved for P_i 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 P_i-starved cells. Through a modulation of the metabolism during P_i 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 ($O_2^{\bullet -}$). Superoxide and the resulting other reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$) 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 H_2O_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 (SodA, SodB), catalases (KatE, KatG), peroxidases (AhpCF, Tpx), DNA-protecting compounds (Dps, polyamines), and DNA-repair enzymes specific for either oxidized bases (XthA, Nfo, Nth and MutMTY for oxidized purines) or DSB (RecBCD, RecA, RecG, RuvABC) (Demple & 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 *sodA* and *nfo*, which responds to redox-cycling drugs; the OxyR regulon, including *katG* and *dps*, which is induced in actively growing cells in response to H_2O_2-mediated oxidation; the RpoS (σ^S) regulon, including also *katG* and *dps*, which is induced when bacteria enter stationary phase; and the LexA regulon, including *recA and ruvAB*, which is induced by DNA damage (Sak *et al*., 1989; Altuvia *et al*., 1994; Hengge-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 aerobiosis to anaerobiosis (Nyström, 1994). For example, fermentative enzymes such as the pyruvate formate-lyase (Pfl) (Böck & Sawers, 1996) are produced in increased amounts, whereas aerobic enzymes such as the NAD-dependent lipoamide dehydrogenase subunit (Lpd) present notably in the pyruvate dehydrogenase complex (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 phospate (P_i) maintain an aerobic metabolism. First, at the onset of P_i starvation, the rate of synthesis of the AceF subunit of the aerobic enzyme PDH (AceEF-Ldp) is not significantly affected, while the rate of synthesis of the fermentative enzyme 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 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 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 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 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 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*(Am) 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*(Am) 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

Strain	Genotype	Source
ENZ361	F⁻ *thr-1*(Am) *ara-14 leuB6* Δ(*argF–lac*)*U169 tsx-33 glnV44* (*supE44*) *galK2 sulA211* rac⁻ *hisG4*(Oc) *rfbD1 mgl-51 rpoS396*(Am) *rpsL31 kdgK51 xyl-5 mtl-1 argE3*(Oc) *thi-1*	Dri & Moreau (1993)
ENZ376	λ*cI*Ind⁻ p*sulA*::*lacZ* lysogen of ENZ361	Dri & Moreau (1993)
ENZ408	As ENZ361 but *hns-205*::Tn*10*	Higgins *et al.* (1988)*
ENZ409	As ENZ376 but *hns-205*::Tn*10*	Dri & Moreau (1993)
ENZ616	As ENZ361 but *lexA71*::Tn*5*(Def)	Krueger *et al.* (1983)*
ENZ618	As ENZ361 but Δ(*srl recA*)*306*::Tn*10*	Willis *et al.* (1981)*
ENZ625	As ENZ361 but *recG263*::*kan*	Mandal *et al.* (1993)*
ENZ644	As ENZ361 but *recO1504*::Tn*5 recF332*::Tn*3*	Kolodner *et al.* (1985); Blanar *et al.* (1984)*
ENZ678	As ENZ361 but *recB268*::Tn*10*	Lloyd *et al.* (1987)*
ENZ679	As ENZ376 but *recB268*::Tn*10*	Lloyd *et al.* (1987)*
ENZ701	As ENZ361 but *malB45 zja-505*::Tn*10 lexA3*(Ind⁻)	Ossanna & Mount (1989)*
ENZ702	As ENZ361 but *ruvA60*::Tn*10*	Sharples *et al.* (1990)*
ENZ720	As ENZ361 but *sup-720*	Spontaneous suppressor
ENZ725	As ENZ361 but *srl-300*::Tn*10*	Willis *et al.* (1981)*
ENZ766	As ENZ361 but *rpoS359*::Tn*10* (null)	Lange & Hengge-Aronis (1991)*
ENZ768	As ENZ361 but *hns*::*neo*	Yamada *et al.* (1991)*
ENZ833	As ENZ361 but *ruvA60*::Tn*10 recG263*::*kan*	Sharples *et al.* (1990); Mandal *et al.* (1993)*
ENZ840	As ENZ720 (*sup-720*) but *nadA57*::Tn*10*	Singer *et al.* (1989)*
ENZ849	As ENZ720 (*sup-720*) but *srl-300*::Tn*10*	Willis *et al.* (1981)*
ENZ858	As ENZ361 but Arg⁺	Spontaneous revertant
ENZ862	As ENZ858 but Thr⁺	Spontaneous revertant
ENZ868	As ENZ862 but His⁺	Spontaneous revertant
ENZ875	As ENZ858 (Arg⁺) but *srl-300*::Tn*10*	Willis *et al.* (1981)*
ENZ878	As ENZ868 (Arg⁺ Thr⁺ His⁺) but *srl-300*::Tn*10*	Willis *et al.* (1981)*
ENZ885	As ENZ361 but *adiA*::MudI1734(kan)	Stim-Herndon *et al.* (1996)*
ENZ897	As ENZ361 but *nadA57*::Tn*10 sup-720*	ENZ840*
ENZ898	As ENZ361 but *nadA*::Tn*10 lysT*(SuUAA/G) (*supG*)	Prather *et al.* (1983)*
ENZ963	As ENZ862 (Arg⁺ Thr⁺) but *srl-300*::Tn*10*	Willis *et al.* (1981)*
ENZ1214	As ENZ361 but *astB*::kan	Schneider *et al.* (1998)*
ENZ1215	As ENZ361 but *astC*::kan	Schneider *et al.* (1998)*
MG1655	F⁻ *rph-1 rfb-50*	M. Cashel, NIH, Bethesda, MD, USA

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

assay ($H_2O_2 \rightarrow O_2 + H_2O$) 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 O_2 from H_2O_2 dropped on bacteria, whereas introduction of the *rpoS*⁺ allele from strain MG1655 (Bachmann, 1996) did further enhance the catalase activity, as shown by an increase in the global production of O_2, 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*::Tn*10* 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 Tn*10* 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 &

Söll, 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 NH_4Cl, 5 mM K_2HPO_4 and 20·2 mM glucose (0·4 %, w/v) supplemented with five vitamins (0·02 mM thiamin, 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 (0·4 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 P_i-limiting medium, the concentration of K_2HPO_4 was reduced from 5 to 0·1 mM, but 9·8 mM KCl was added to maintain the concentration of potassium as in MOPS medium (Dri & Moreau, 1993); in glucose-limiting medium, the concentration of glucose was reduced from 0·4 to 0·04 or 0·05 % (w/v), as indicated. $MOPS_0$ buffer was MOPS medium deprived of ammonium, phosphate, glucose, amino acids and vitamins. $M9_0$ buffer consisted of 3 g KH_2PO_4, 7·5 g $Na_2HPO_4.2H_2O$, 1 g NH_4Cl and 5 g NaCl per litre. Minimal agar medium (Miller, 1972) was $M9_0$ supplemented with 1 mM $MgSO_4$, 0·1 mM $CaCl_2$, 0·03 mM thiamin, 0·2 % (w/v) glucose, amino acids when required (0·4 mM threonine, 0·8 mM leucine, 0·2 mM histidine, 0·4 mM arginine, 0·4 mM isoleucine, 0·6 mM valine) and 12 g granulated agar l^{-1}. LB agar medium (Miller, 1972; Difco) contained 10 g Bacto tryptone, 5 g Bacto yeast extract, 10 g NaCl and 15 g Bacto agar l^{-1}. Media were supplemented with 10 μM niacinamide for growing *nadA* transductants. 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 $MOPS_0$ buffer, diluted 1:200 into 50 ml MOPS medium limited in P_i 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 $M9_0$ 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 within one log at each time point.

Mixed-culture experiments. Of the two strains used, one (generally that used as the minor population) carried a Tn*10* (Tc^r) antibiotic-resistance marker. Unless otherwise indicated (Fig. 3d), Tc^r and Tc^s 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_i (time zero), grown for 24 h, and mixed (day 1) by transferring 50 μl of a 1:100 dilution in $MOPS_0$ 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 $M9_0$, and plated in triplicate onto LB and LB-tetracycline agar media to determine the total number of viable cells and the number of Tc^r 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 Arg^+, His^+ and/or Thr^+ revertants, more variations were observed and data from several independent experiments are shown.

Growth in spent culture medium. Cultures were grown in P_i-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 $MOPS_0$, 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 OD_{600} 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): $[(OD_{420} - 1{\cdot}75\,OD_{550}) \times \text{reaction volume} \times 1000]/(\text{reaction time} \times \text{sample volume} \times OD_{600})$; OD_{420} and OD_{550} 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 P_i-starved cells

The possibility that *E. coli* cells starved for P_i 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), *hisG4*(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 P_i or glucose (R. Duval & P. L. Moreau, unpublished results),

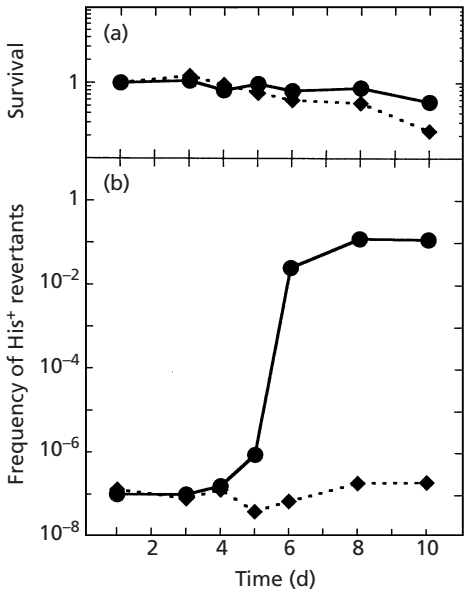

Fig. 1. Accumulation of prototrophic revertants during prolonged incubation in P_i-limiting medium. *E. coli* ENZ361 cells were grown to saturation in MOPS medium ($2{\cdot}5 \times 10^9$ cells ml^{-1}), centrifuged, resuspended in the same volume of $MOPS_0$ buffer, diluted 1:200 (designated time zero) 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 ($6{\cdot}2 \times 10^8$ and $5{\cdot}5 \times 10^8$ 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.

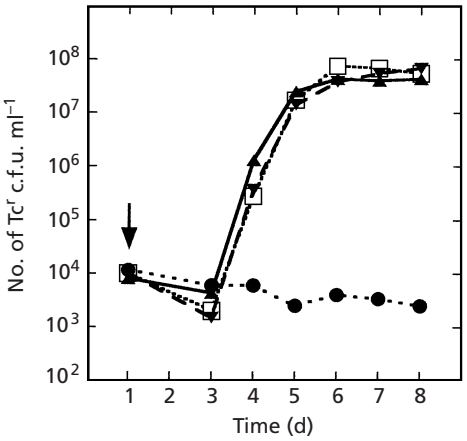

Fig. 2. Growth of *supG* suppressors in mixed culture. Strains ENZ725 (*srl*::Tn*10*) (●), ENZ849 (*sup-720 srl*::Tn*10*) (□), ENZ897 (*nadA*::Tn*10 sup-720*) (▼) and ENZ898 (*nadA*::Tn*10 supG*) (▲) were grown for 1 d in P_i-limiting medium, and rediluted $1:10^5$ into 1-d-old cultures of strain ENZ361 (Tc^s) 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 6×10^8 ml^{-1} on day 1 to about 4×10^8 cells ml^{-1} on day 8 of incubation. Data are the means of two independent experiments.

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 *nadA57*::Tn*10* 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*::Tn*10* (Tc^r) 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^r suppressors started to grow after 2 further days of incubation, and reached a maximal concentration of about 10^8 cells ml^{-1} after 3 more days of incubation, while the total number of viable cells was about 4×10^8 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 Tn*10* (Tc^r) 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

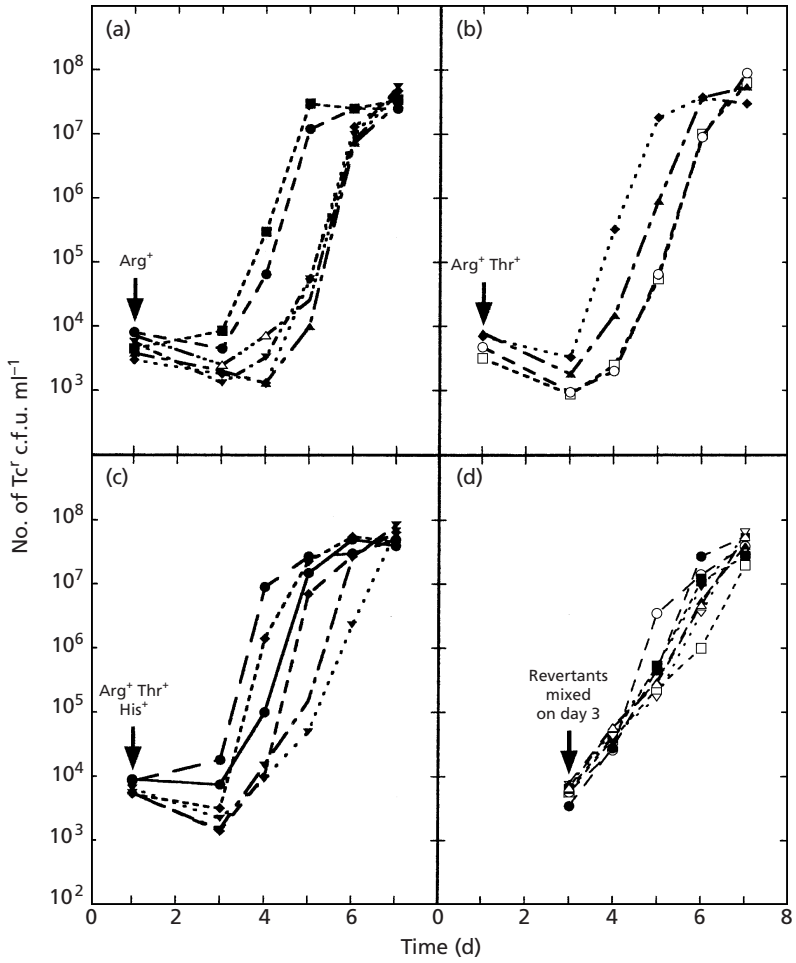

Fig. 3. Growth of Arg$^+$ revertants in mixed culture. In (a), (b) and (c), mixed-culture experiments were done with ENZ875 (Arg$^+$ *srl*::Tn*10*) (a), ENZ963 (Arg$^+$ Thr$^+$ *srl*::Tn*10*) (b) and ENZ878 (His$^+$ Arg$^+$ Thr$^+$ *srl*::Tn*10*) (c) revertants grown for 1 d in P_i-limiting medium and then rediluted 1:10^5 into 1-d-old cultures in P_i-limiting medium of strain ENZ361 (Tc^s) containing about $6{\cdot}2 \times 10^8$ cells ml^{-1}. In (d), 1-d-old Arg$^+$ Tc^r (■, □), Arg$^+$ Thr$^+$ Tc^r (▼, ▽) and Arg$^+$ Thr$^+$ His$^+$ Tc^r (●, ○, ▲, △) revertants were rediluted 1:10^5 into 3-d-old cultures in P_i-limiting medium of strain ENZ361 (Tc^s) containing about $4{\cdot}1 \times 10^8$ cells ml^{-1}. By day 7, the total number of viable cells in cultures was about $3{\cdot}6 \times 10^8$ cells ml^{-1}. Data of several independent experiments are shown.

minor or as the major population in mixed cultures (data not shown), and introduction of the *srl*::Tn*10* 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

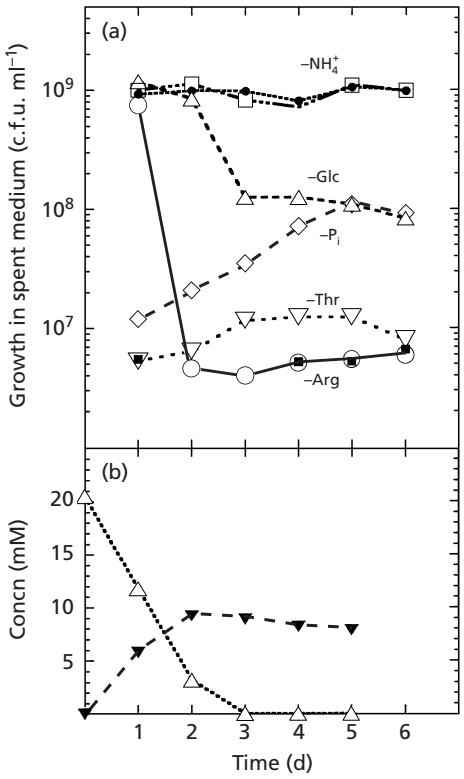

Fig. 4. Concentrations of nutrients in spent culture media. ENZ361 cells grown overnight in glucose-limiting medium were diluted 1:200 in P_i-limiting medium (time zero) and grown further with aeration. (a) At the times indicated, samples were withdrawn, sterilized by filtration and inoculated at a final concentration of about $6{\cdot}5 \times 10^6$ cells ml^{-1} with ENZ361 cells starved for glucose. Media were either not supplemented (■), or supplemented with five nutrients (10 mM NH_4Cl, 5 mM K_2HPO_4, 10 mM glucose, 0·4 mM threonine and 0·4 mM arginine) (●), or with only four nutrients, one nutrient being omitted: NH_4Cl (□), glucose (△), P_i (◇), 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.

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 P_i in the culture medium appeared to increase steadily during the first 5 d of incubation (Fig. 4a; curve $-P_i$), which indicates that P_i was actually excreted by non-growing bacteria. It is known that cells entering stationary phase under P_i-starvation conditions maintain high intracellular P_i levels (about 7 mM) (Rao *et al*., 1993; N. W. Lutz & P. L. Moreau, unpublished results), which provides a potential source of P_i. Like P_i, 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 P_i-starved cells. At first glance, it seemed that bacteria starved for P_i 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 P_i-starved cells can totally degrade glucose to products such as acetate. Likewise, arginine appeared to be used slowly but extensively by P_i-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 $-NH_4^+$), and inactivation of *astB* or *astC* had no effect on the rate of degradation of arginine during P_i-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 (SpeA)-initiated pathway to produce agmatine and putrescine because putrescine, which is a weak inhibitor of SpeA activity (Glansdorff, 1996), preferentially delayed the degradation of arginine when it was added into P_i-limiting medium to a final concentration of 10 mM (data not shown).

Taken together, these results show that ENZ361 cells starved for P_i 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 P_i, acetate and threonine, that are progressively excreted by the bulk of the population.

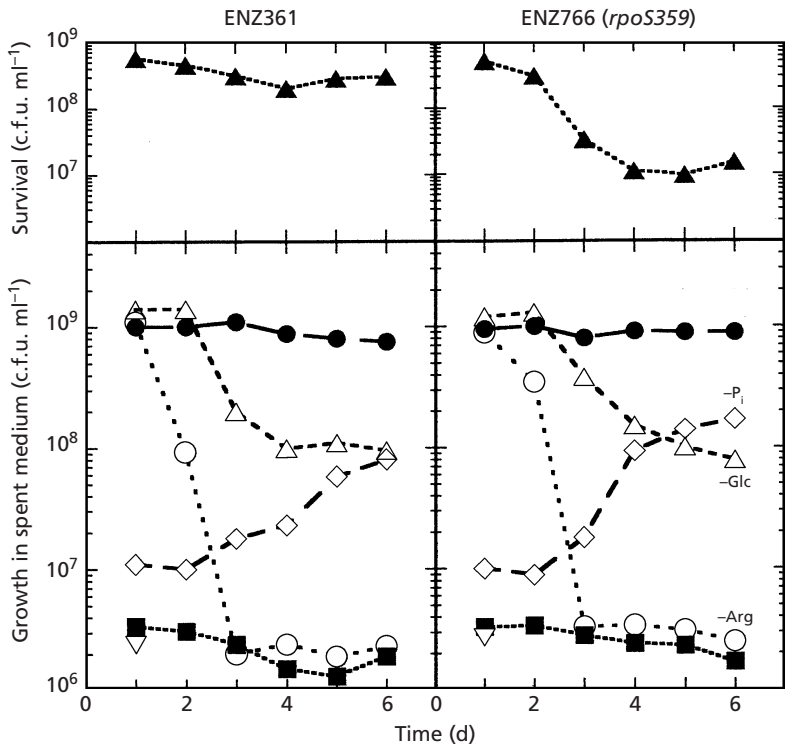

Fig. 5. Role of RpoS. Experiments were done as described in the legend to Fig. 4 except that each culture in P_i-limiting medium was inoculated directly with a single colony grown on LB medium (bacteria were not pre-grown in MOPS medium limited in glucose to avoid the production of secondary mutations in *rpoS* mutants). Survival (measured on LB medium) of strains ENZ361 and ENZ766 (*rpoS359*::Tn*10*) during prolonged incubation in P_i-limiting medium is shown in the top panels. At the times indicated, samples were withdrawn, sterilized by filtration and inoculated with ENZ361 cells starved for glucose at a final concentration of about 3×10^6 cells ml^{-1}. Media were either not supplemented (■), or supplemented with four nutrients (5 mM K_2HPO_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 (○). The reconstituted cultures were incubated for 24 h and the concentrations of viable cells were determined on LB medium (lower panels). The data are from a representative experiment from three separate trials. By day 6, the concentrations of acetate in cultures of strains ENZ361 and ENZ766 (*rpoS359*::Tn*10*) were, respectively, 7·3 and 7·2 mM.

Protective role of the RpoS regulon during P_i starvation

Because *supG* suppressors rather than Arg^+ revertants were found in old cultures starved for P_i (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, P_i starvation strongly induces the expression of the *rpoS* gene (Lange & Hengge-Aronis, 1991), and P_i-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 P_i.

To assess the possible role of RpoS during P_i 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 P_i-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 P_i-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 P_i-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 P_i-starvation conditions (see Fig. 7a, b).

Recombinational repair of DSB in P_i-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 P_i prompted us to test the role of LexA-controlled DNA repair genes (Walker, 1996), which are also induced under P_i-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 P_i, whereas inactivation of other DNA repair genes, such as *uvrA*, whose product catalyses excision of

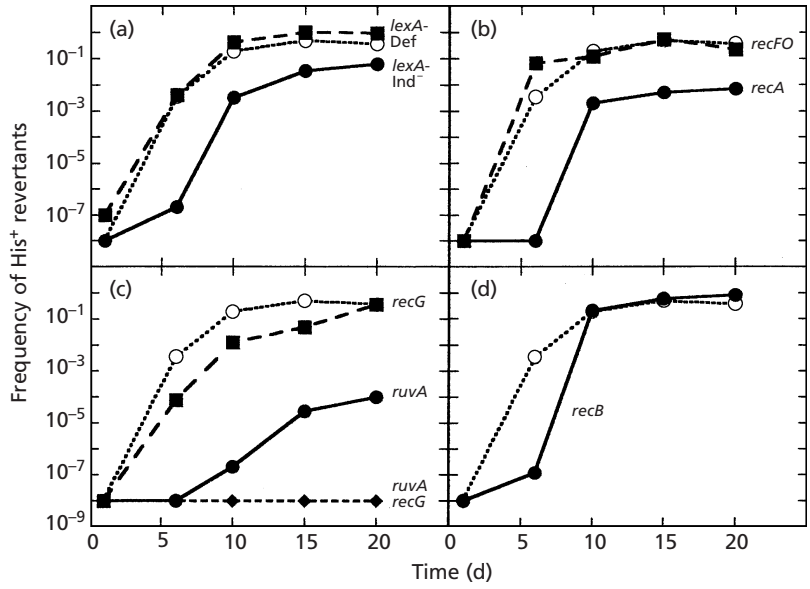

Fig. 6. Altered ability of recombination mutants to accumulate prototrophic revertants during prolonged incubation in P_i-limiting medium. Experiments were done as described in the legend to Fig. 1. For each strain, the total number of viable cells measured on day 1 of incubation is indicated in parentheses. (a) ENZ616 (*lexA*-Def) ($4{\cdot}7 \times 10^8$ cells ml^{-1}) (■) and ENZ701 (*lexA*-Ind$^-$) ($5{\cdot}6 \times 10^8$ cells ml^{-1}) (●); (b) ENZ618 (*recA*) ($3{\cdot}6 \times 10^8$ cells ml^{-1}) (●) and ENZ644 (*recFO*) ($4{\cdot}1 \times 10^8$ cells ml^{-1}) (■); (c) ENZ625 (*recG*) ($4{\cdot}7 \times 10^8$ cells ml^{-1}) (■), ENZ702 (*ruvA*) ($2{\cdot}5 \times 10^8$ cells ml^{-1}) (●) and ENZ833 (*ruvA recG*) ($8{\cdot}5 \times 10^7$ cells ml^{-1}) (◆); and (d) ENZ678 (*recB*) (●) ($2{\cdot}2 \times 10^8$ cells ml^{-1}). Values obtained with parental strain ENZ361 ($6{\cdot}6 \times 10^8$ 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 P_i-starved cells

Each strain was grown overnight in glucose-limiting medium, diluted 1:200 into 50 ml P_i-limiting medium (time zero) in a 500 ml Erlenmeyer flask, and grown further with aeration. At an OD_{600} of about 0·25 (exponential growth phase), a sample was withdrawn from each culture and split into three parts: one part was left untreated; one part was UV-irradiated (approx. 7·5 J m^{-2}), brought to 2 mM K_2HPO_4 and further incubated for 1 h; and one part was supplemented with 25 μg nalidixic acid (Nal) ml^{-1} and 2 mM K_2HPO_4 and further incubated for 2 h. The specific activity of β-galactosidase was then determined in each portion. The cultures in P_i-limiting medium were further incubated for 28 h and assayed for β-galactosidase activity as described in Methods. The OD_{600} values of the cultures after 28 h incubation were about 0·72, 0·77 and 0·58 for strains ENZ376, ENZ409 and ENZ679, respectively. The values are the means (± SD) of three (strain ENZ376) or four (strains ENZ409 and ENZ679) independent experiments.

Strain	β-Galactosidase activity			
	Exponential growth			P_i starvation 28 h
	Untreated	+UV	+Nal	
ENZ376	137 ± 19	2840 ± 457	3360 ± 385	1328 ± 31
ENZ409 (*hns-205*)	212 ± 32	2207 ± 345	2322 ± 386	501 ± 100
ENZ679 (*recB*)	30 ± 5	842 ± 278	36 ± 2	49 ± 2

bulky lesions, had no effect (data not shown), which suggests that recombinational repair may protect P_i-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 P_i-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 P_i-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

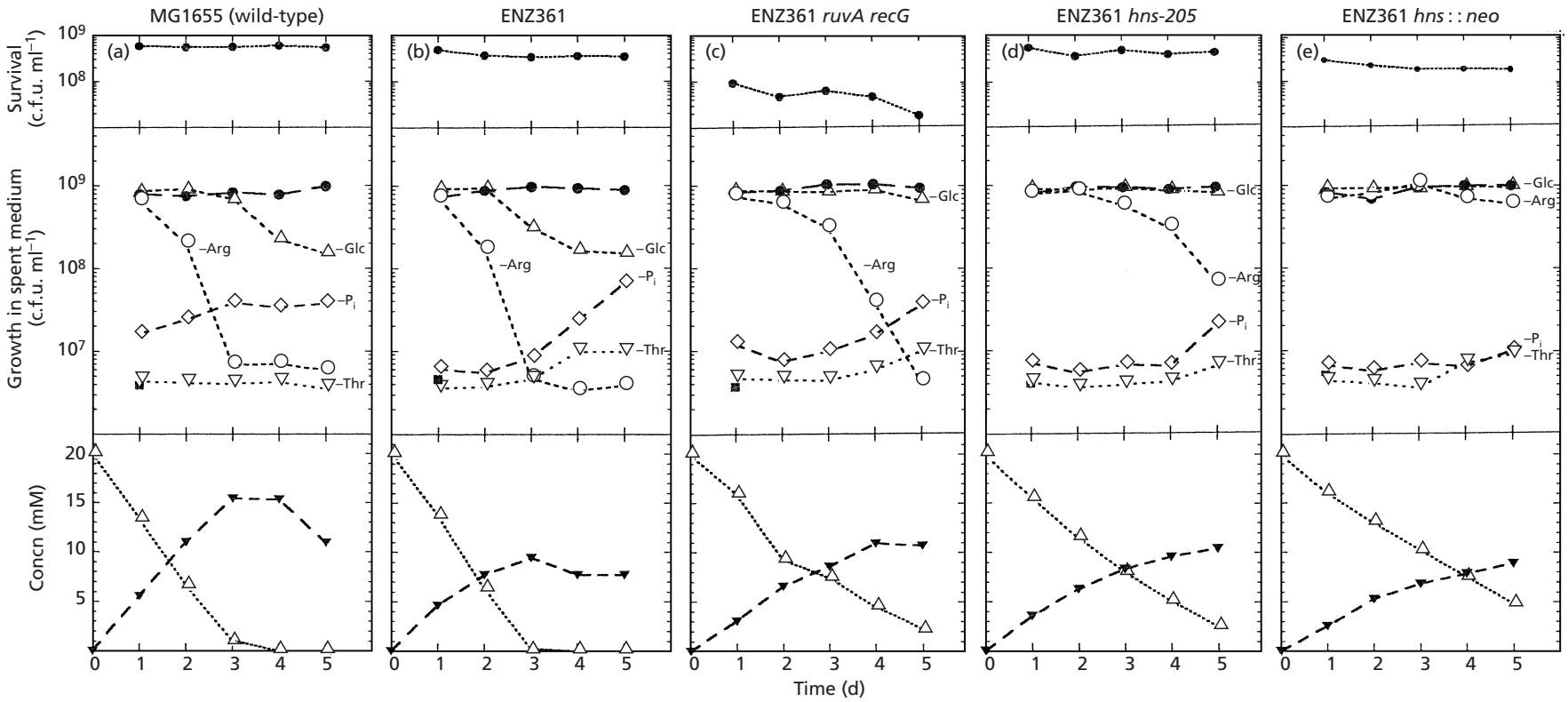

Fig. 7. Effects of *ruvA recG* and *hns* mutations on viability and metabolism under P_i-starvation conditions. Experiments were done as described in the legend to Fig. 5 with strains (a) MG1655 (wild-type), (b) ENZ361, (c) ENZ833 (ENZ361 *ruvA recG*), (d) ENZ408 (ENZ361 *hns-205*::Tn*10*) and (e) ENZ768 (ENZ361 *hns*::*neo*). Spent media were either not supplemented (■), or supplemented with four nutrients (5 mM K_2HPO_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.

(Lloyd & Low, 1996; Cox, 1997). 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 P_i, (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 P_i 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 & Demple, 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 (Dri & 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*::Tn*10* 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, Fig. 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 anaerobic (Pfl) or semi-anaerobic (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,

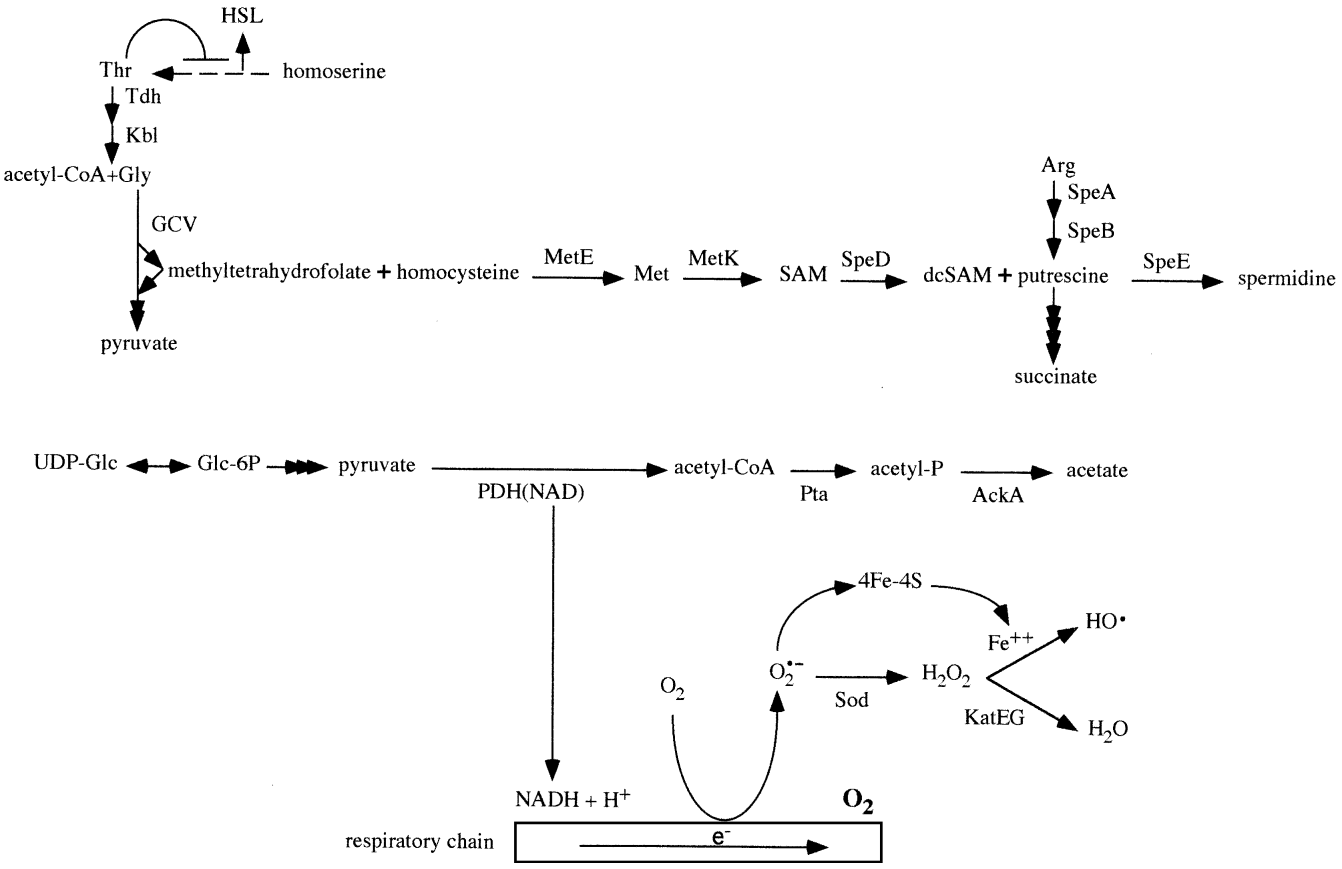

Fig. 8. Diagram showing the proposed pathways for the degradation of threonine, arginine and glucose, and for the production of ROS, in P_i-starved cells. H-NS may inhibit the synthesis of Tdh and Kbl, and thus may enhance the synthesis of homoserine lactone (HSL); on the other hand, H-NS may enhance the synthesis of PDH (AceEF-Lpd), thereby increasing the metabolism of glucose to acetate. Because UDP-Glc inhibits RpoS activity, whereas HSL and acetate enhance RpoS activity, H-NS may globally increase RpoS activity which in turn increases the activity of the catalases KatE and KatG. GCV, glycine cleavage system (GcvHPT-Lpd); dcSAM, decarboxylated *S*-adenosylmethionine. See Discussion for details.

excess-glucose conditions do (El-Mansi & Holms, 1989; Holms, 1996).

If the above conclusion is correct, P_i-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 P_i, 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 P_i-starved cells. The notion that P_i-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 P_i-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 P_i-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 P_i-starved cells is totally prevented by introduction of a *recB* mutation clearly indicates that DSB are the main DNA damage produced during starvation for P_i.

The requirement of RpoS-dependent functions for survival of P_i-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 P_i-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 P_i-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 (Satishchandran *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 aggression 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 ($E\sigma^s$) 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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