Acta Biochimica Polonica

Vol. 53 No. 4/2006, 729–738

on-line at: www.actabp.pl

This article is dedicated to our late mentor Professor Michel Blot

Regular paper

IS*1* transposition is enhanced by translation errors and by bacterial growth at extreme glucose levels⋆

Arun S. Kharat✉, Evelyne Coursange, Marjolaine Noirclerc-Savoye, Jérôme Lacoste and Michel Blot

Plasticité et Expression des Génomes Microbiens, CNRS-FRE2383/CEA LRC12/Université Joseph Fourier, Grenoble, France ; ✉e-mail: aru@teacher.com

Received: 12 April, 2006; revised: 16 August, 2006; accepted: 04 October, 2006
available on-line: 14 November, 2006

Transposition of insertion sequences (IS) is an enzyme-mediated process that only occurs in a minority of cells within a bacterial culture. Transposition is thus a rare event, but transposition frequency may vary depending on experimental conditions. For instance in a rich broth, IS elements are known to transpose during stationary phase but not during exponential growth. Using a reporter system which involves the activation of the cryptic *bgl* operon in *Escherichia coli*, we show that the frequency of IS*1* transposition is a function of glucose concentration in the growth medium, it is increased by streptomycin amounts that are below minimum inhibitory concentration (sub-MIC) and is inhibited in an *rpsL150* strain with high translation accuracy. Since starved cells are known to enhance ribosome frameshifting, our data suggests that growth conditions applied in this study could affect IS*1* transposition by increasing translation infidelity.

Keywords: IS elements, β-glucosides, programmed translation frameshift, *bgl* operon

INTRODUCTION

Transposable elements reside on the chromosomes of all sequenced bacteria and seem ubiquitous among many bacterial species (Mahillon & Chandler, 1998; http://www-is.biotoul.fr/is.html). Systematic studies on the spectrum of mutations have shown that insertion sequences (IS) provide a major contribution to spontaneous mutagenesis (Rodriguez *et al*., 1992; Hall, 1999a). The direct impact and the evolutionary role of IS elements have been extensively discussed because they were discovered as detrimental insertions, but they were also shown to generate mutations enhancing fitness (Chao *et al*., 1983; Chao & McBroom, 1985; Treves *et al*., 1998; Hall, 1999a; Cooper *et al*., 2001; Schneider *et al*., 2001).

Transposition of mobile genetic elements is an enzyme-mediated process requiring proteins expressed by the element itself (a transposase) and host genes as well. These host proteins include not only a polymerase and a ligase, but also other DNA binding proteins such as IHF, HU, HNS, Fis; the replication initiator DnaA; the protein chaperone/protease ClpX, ClpP, and ClpA; the SOS control protein LexA; and the Dam DNA methylase (Mahillon & Chandler, 1998; Shiga *et al*., 2001), thus regulation of transposition depends on biochemical activities of the host. It has been proposed that transposition rates have evolved to be low enough to avoid a burden on the host genome so that IS elements may represent "not-too-harmful genetic parasites" (Orgel & Crick, 1980). This leads to a paradox at the population level as the transposition in itself is an enzymatically-driven process, but affects only a few individuals among billions of isogenic cells. How much this proportion is random or may depend on the biochemistry of the cells and their environment is a question of primary importance to understand the genetic adaptation to an environment.

Considering bacterial life styles, one can typically distinguish periods of abundance, when a bacterium grows as much as its genomic abilities allow, and periods of nutritional deprivation, when any mutation favouring continued growth would be

⋆Genbank accession numbers: AF312716 and AF312717.

Abbreviations: IS, insertion sequence; MIC, minimum inhibitory concentration; rRNA, ribosomal RNA; Sm, streptomycin.

advantageous. Thus, controlling mutation rates as a function of the growth regimen appears to be a reasonable strategy for the bacteria. For instance, it was shown that IS transposition increases with the length of bacterial starvation on agar plates (Hall, 1999b). IS transposition is not observed during exponential growth in rich media, but mutants become numerous when the medium is depleted or during exponential growth in a carbon-limited minimal medium (Noirclerc-Savoye & Blot, unpublished).

Utilization of β-glucosides in *Escherichia coli* is cryptic: most of the wild type strains exhibit the Arbutin/Salicin/Cellobiose negative ($Arb^-Sal^-Cel^-$) phenotype although at least four genetic systems involved in β-glucoside utilization (including the *arbT* locus, the *bgl*, *asc* and *cel* operons) exist on the chromosome; surprisingly, they all are rendered silent. Spontaneous Arb^+Sal^+ (Bgl^+) mutants are predominantly due to activation of the cryptic *bgl* operon (Reynolds *et al*., 1981). Mutational activation of the *asc* or *cel* operons also confers the Arb^+Sal^+ phenotype, however, the Sal^+ phenotype conferred by *asc* is weak compared to that of *bgl* whereas the *cel* operon confers the Cel^+ phenotype in addition to Sal^+. In contrast, mutations in *arbT* results only in the Arb^+ phenotype (Hall, 1998). The majority of the Bgl^+ mutants are due to transposition of IS elements into the *bglR* region, although mutations in unlinked loci such as *hns*, *gyrA*, *bglJ* and *leuO* can also activate the *bgl* operon (Defez & Felice, 1981; DiNardo *et al*., 1982; Giel *et al*., 1996; Ueguchi *et al*., 1998).

The main purpose of this study was to investigate the role of limiting and excess glucose concentrations in growth medium on transposition of *E. coli* K12 insertion sequences by using the *bgl* operon as a genetic reporter system.

In the study we shall show that (i) limiting or excess glucose concentration alters IS*1* transposition; (ii) provoking translation infidelities increases the rate of IS*1* transposition; and (iii) enhancing translation accuracy inhibits IS*1* insertions into the reporter *bgl* operon, suggesting that growth conditions exert an effect on translation accuracy, and thus on mutability.

MATERIALS AND METHODS

Bacterial strains and growth. Bacteria were grown in minimal medium M9 (Sambrook *et al*., 1989) supplied with different glucose concentrations in 96-well micro-titration plates. Cultures were supplemented when appropriate with streptomycin (Sm) (50 μg/ml), arabinose (1%), and plated on various agar plates including commercial LB agar plates, M9 arbutin (0.1% as the sole carbon source), MacConkey salicin (1%), MacConkey arabinose (1%).

The *E. coli* K-12 strains used in this study were as follows: strain ZK126 (a derivative strain of W3110): λ^-, IN(*rrnD-rrnE*)*1*, *rph1 tna*-2, Δ*lacU169)* (Chao *et al*., 1983); strain MC4100: F^-, *araD139*, Δ(*argF-lac*)*169*; strain LAM^-, *flhD5301*, *fruA25*, *relA1*, *rpsL150*, *rbsR22*, *deoC1*, (Casadaban, 1976); strain GBE150: ZK126 transduced with *rpsL150* (present study); Strain GBE150, same as ZK126 but carrying *rpsL150* mutation in the *rpsL* gene.

Construction of GBE150 strain carrying the *rpsL150* mutation. Strain GBE150 was constructed by P1-transduction, in which the P1 phage was grown on the Ara^-Str^R donor MC4100 and P1 lysate obtained was transduced to the Ara^+ Str^S recipient ZK126. Primary transductants were selected on LB agar plates supplied with 50 μg/ml streptomycin and 5 mM sodium citrate. One of the Str^R Ara^+ transductant was referred to as GBE150 and used to confirm the presence of *rpsL150* mutation by DNA nucleotide sequencing. In brief, oligonucleotide primers used to amplify the *rpsL* gene from the genomic DNA of MC4100, ZK126 and GBE150 were 5′-acgttttattacgtgtttacg-3′; 5′-cgtttggccttacttaacgg-3′. PCR products were purified from the gel and sequenced from both ends. The strain was confirmed to carry *rpsL150* mutation and was then used to study the role of the translation fidelity on transposition.

Isolation of Bgl^+ mutants and fluctuation test. In each experiment a set of 45 to 55 replicate cultures was made in M9 medium supplemented with 0.4% glucose (0.3 ml with about 10^3 cells) in capped microtitration plates (96 wells). The culture set was incubated at 37°C with shaking (160 r.p.m.) until the OD_{600} reached the value of 0.8 at which time 0.1 ml of each culture was plated on M9-arbutin. The cultures were then re-incubated for an additional period of 14.5 h during which the cultures entered in the stationary phase of growth. Another set of 0.1 ml aliquots were then removed from each culture and these were also plated on M9-arbutin. Plates from both the exponential and stationary phase were incubated at 37°C for 48 h for the appearance of Bgl^+ mutants. Incubation of the plates was restricted to 48 h to prevent "post-plating" mutants (Hall, 1998). The plates from exponentially growing culture without any mutants were considered for studying the stationary phase-specific mutants, and the proportion of plates without colonies was used to estimate the mutation rate using the method of Lea and Coulson (1949). Nalidixic acid (Nal^R) mutants were isolated in the same manner as described above. They were used as controls to determine whether the altered glucose conditions had any effect on the rate of point mutations in the housekeeping gene *gyrA*.

Study of the *bglR* region. The primers used to amplify the *bglR* region were selected on the basis of the wild type *E. coli bgl* sequence (Schnetz *et*

al., 1987): 5′-cgatgagctggataaactgc-3′;5′-cgctgttcatcatcaataacc-3′. The size of the PCR products distinguished insertion from non-insertion mutants. Insertion mutants were then processed for Southern analysis with internal probes of IS elements (Schneider *et al*., 2000). Probing with the IS*1*, IS2, and IS*5* elements was sufficient to characterize all insertion mutants in this study.

Real-time quantitative PCR. Real-time PCR reactions were performed by following suggestions made by the manufacturer. Oligonucleotide primers used to amplify IS*1* were 5′-tctccggaagacggttgttg-3′ (F) and 5′-ccccagcgtggccata-3′ (R) (within *insB*) and 16S rRNA 5′-cgtgttgtgaaatgttgggttaa-3′ (F) and 5′-ccgctggcaacaaaggatta-3′ (R). In brief, a 25 μl reaction mixture contained 1 × master mix supplied with SYBR Green I Dye (Eurogenetec), 2 μm of each primers, and 50 ng of cDNA synthesized from DNA -free purified RNA used in reaction above, PCR amplifications (2′ at 50° — 10′ at 95° — 40 × [15′′ at 95° — 1′ at 60°]) were carried out in a GenAmp 5700 sequence detection system. Specific IS*1* transcription was deduced by the ratio of IS*1* to 16S rRNA, and the relative amount of IS*1*-mRNA in different glucose conditions was normalised to one unit of the value at 0.4% (standard) glucose.

RESULTS

Bacterial growth in various glucose concentrations

The ZK126 strain was tested for growth in M9 medium supplemented with a range of concentrations of glucose as a sole carbon source. Growth at 0.4% glucose gave a generation time of 90 min. Although at 4.0% glucose the cells grew faster, with a generation time of 70 min, the viable cell number of this culture was found to drop during the stationary phase by an order of magnitude. For this reasonthe 0.4% glucose concentration is referred to as the "standard" and 4% as an "excess" glucose concentration in our studies. However, 4% glucose was still very useful for studies of transposition.

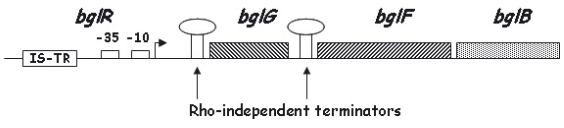

Figure 1. Schematic representation of the *bgl* operon. A unique promoter controls three genes: *bglG (*positive regulator acting by anti-termination of Rho-independent structures), *bglF* (PEP-dependent phosphotransferase transporting aryl-β-glucosides and also negative regulator by phosphorylation of BglG in absence of β-glucosides) and *bglB* (phospho-β-glucosidase). The *bglR* region located upstream to the promoter is the regulatory element. The box designated as IS-TR indicates a 49 bp target region for the transposition of IS elements. The boxes –10 and –35 indicate location of the promoter. An arrow shows the transcription start site. Rho-independent terminators are depicted as a stem loop structure.

Based on 1790 independent cultures, mutation rates were estimated before (exponential phase) and after (stationary phase) the cultures had reached the OD_{600} = 0.8. The average mutation rate was 2.7×10^{-8} mutation per cell per generation during exponential growth and 2.0×10^{-8} per cell generation during stationary phase.

The *bgl* operon reporter genetic system: isolation and characterization of Bgl$^+$ mutants

The *bgl* operon (Fig. 1) of *E. coli* is a well studied cryptic genetic system that contains the genetic machinery for the uptake and metabolism of aryl β-glucosides such as arbutin and salicin. Wild type *E. coli* strains exhibit a Bgl$^-$ phenotype and spontaneous mutants with the Arb$^+$Sal$^+$ phenotype can be obtained on MacConkey Arbutin or MacConkey Salicin plates. Activating mutations can be linked or unlinked to the *bgl* operon. The majority of the linked mutations are due to transposition of insertion sequences into a regulatory element, the *bglR* region (Reynolds *et al*., 1981; Schnetz & Rak, 1992) whereas mutations in unlinked loci such as *hns,* gyrase, *bglJ* and *leuO* result in activation of the *bgl* operon (Defez & Felice, 1981; DiNardo *et al*., 1982; Giel *et al*., 1996; Ueguchi *et al*., 1998). Since most of the Bgl$^+$ mutants are a result of transposition events the activation of *bgl* operon by IS elements is assumed to be a good index of their mobility (Reynolds *et al*., 1981; Rodriguez *et al*., 1992; Schnetz & Rak, 1992; Moorthy & Mahadevan, 2002). The Arb$^+$ mutations could be due to not only activation of the *bgl* operon but also to mutations in the *arbT* locus (Arb$^+$Sal$^-$), activation of the *asc* operon giving a weak Sal$^+$ phenotype, and activation of the *cel* operon giving the Arb$^+$Sal$^+$Cel$^+$ phenotype. Mutations that were Sal$^-$ (*arbT* locus), weak Sal$^+$ (*asc* operon) or Cel$^+$ (*cel* operon) were eliminated from the study and only Arb$^+$Sal$^+$ (*bgl* operon) mutants were used in further analysis. The Bgl$^+$ mutants described above were isolated from the ZK126 and GBE150 strains of *E. coli* K12.

A total of 792 independent Bgl$^+$ mutants were identified from standard, limiting and excess glucose concentrations during exponential and stationary phase growth. As the aim of this study was to investigate the role of limiting and excess glucose concentrations on the rate of transposition of IS elements, mutants displaying wild type PCR product in the *bglR* region (398 bp) (i.e. PCR products without insertions) were not used in further investigation. Insertional mutants displaying larger PCR products (760 to 1450 bp) were used in Southern hy-

Table 1. Mutation rates and mutation frequencies at different glucose concentrations for the wt (ZK126) and *rpsL* (GBE150) strains

Strain	Glucose (%)	Doubling time (min)	No. of cultures tested	No. of plates without mutants	Mutation frequency 10^{-7}	Mutation rate[a] 10^{-8}mut./(cell × gen.)
During exponential phase						
ZK126	0.1	240	225	107	1.6	6.4
ZK126	0.4	90	200	129	1.0	0.8
ZK126	4	70	90	17	12.3	1.7
GBE150	0.1	300	135	72	1.7	4.9
GBE150	0.4	120	180	121	0.8	0.5
GBE150	4	100	90	17	11.7	1.9
During stationary phase						
ZK126	0.1	–	225	170	0.6	0.4
ZK126	0.4	–	245	176	0.2	0.5
GBE150	0.1	–	200	150	2.8	1.6
GBE150	0.4	–	200	160	0.2	0.3

[a]According to Lea & Coulson (1949). Analysis of the Z-test on the data indicated mutation rates were significantly increased at extreme glucose ($P < 0.05$) whereas the differences of mutation rate in ideal conditions but in two genetic backgrounds (wt and *rpsL150*) were not statistically significant ($P < 0.05$).

bridizations and were identified as IS*1*, IS*2* and IS*5* insertions. Of the 792 Bgl$^+$ mutants, 223 were due to transposition of an IS element, either IS*1*, IS*5* or IS*2* whereas the remaining 569 mutants were from non-IS insertion and could be due to point mutations in the CAP-cAMP binding box or due to mutations in unlinked loci. Amongst the 223 mutants carrying an IS element within the *bglR* region, the great majority of these were insertions of IS*1* and in a few cases they were either IS*2* or IS*5*.

Glucose concentration and IS*1* transposition

Bacterial growth in liquid M9 cultures is limited by the concentration of glucose (Fig. 2). After preliminary trials, three concentrations were selected for the fluctuation tests: 0.4% as the "standard" glucose concentration, 0.1% as "limiting" and 4%as "excess" glucose concentration. When compared with the standard glucose concentration, the mutation rate during exponential growth increased for strain ZK126 by 2–3 fold in the presence of excess glucose and 8–9 fold in the presence of limiting glucose concentrations (Table 1). A Z-test analysis of mutation rates indicated that these data were statistically significant ($P < 0.05$). A similar statistical analysis of mutant frequencies indicated that only the data with the excess glucose concentration were significant ($P < 0.05$). During the stationary phase, cultures growing with excess glucose concentration exhibited a reduced viability count by one log unit and therefore the effect of excess glucose concentration on the mutation rate could not be estimated for this sample.

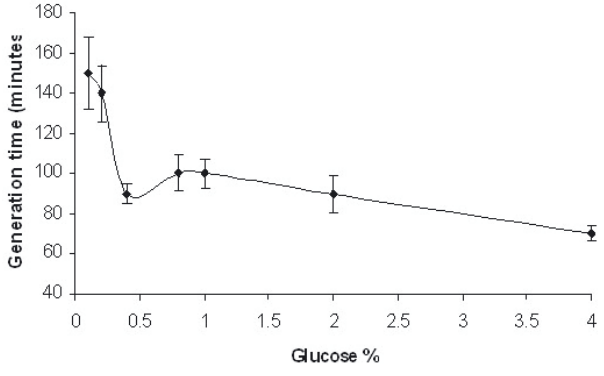

Figure 2. Generation time in M9 as a function of glucose concentration (0.04%, 0.1%, 0.2%, 0.4%, 0.8%, 1%, 2%, and 4%).
Generation time was measured with ZK126 during the linear part of the exponential phase as the doubling time (three replicates). Growth in micro-titration plates is slower than that of the test tube culture because the agitation is restricted.

To test if the mutation rates for spontaneous mutations are increased or decreased by different glucose concentrations, fluctuation analysis was carried out by isolating mutants of nalidixic acid resistant (Nal^R) phenotype, which is due to point mutations in the A subunit of DNA gyrase. Results obtained showed that the mutation rates for the Nal^R phenotype for bacteria growing with different glucose concentrations were fairly similar (not shown), suggesting that the varied mutation rates for Bgl$^+$ mutants were likely due to altered physiology under these growth condition.

The proportion of IS*1* transposition varied with the glucose concentration in the M9 medium (Table 2). The reference value for comparisons was 24% in the standard (0.4%) glucose grown ZK126 during exponential growth. The proportion of IS*1* insertions into the *bglR* region increased up to 36% with limited glucose (0.1%), and it increased to 83% with excess glucose (4%) concentration. A parametric Chi-square analysis on IS*1* *versus* non IS*1* mutations indicated that the increase of IS*1* transposition observed with limiting and excess glucose concen-

Table 2. Distribution of IS insertions at different glucose concentrations in ZK126 (wt) and GBE150 (*rpsL150*)

Strain	Glucose concentration (%)	Doubling time (min)	Number of mutants analyzed	Insertions (%)			Non insertions (%)
				IS*1*	IS*5*	IS*2*	
Exponential phase							
ZK126	0.1	240	118	36	6	0	58
ZK126	0.4	90	71	24	3	0	73
ZK126	4.0	70	73	83	3	0	14
GBE150	0.1	300	63	2	5	0	93
GBE150	0.4	120	59	0	0	0	1
GBE150	4.0	100	73	1	52	3	44
Stationary phase							
ZK126	0.1	–	55	18	4	0	78
ZK126	0.4	-	69	22	4	0	74
ZK126	4.0	-	81	7	5	0	88
GBE150	0.1	-	50	0	0	0	100
GBE150	0.4	-	40	4	0	0	96
GBE150	4.0	-	40	2	8	0	90

A Chi-square analysis of mutants with insertion *versus* non insertion of the ZK126 strain during the exponential phase indicated that the increase of IS*1* transposition seen in excess glucose is highly significant at $P < 0.05$.

trations was statistically significant ($P < 0.05$). Compared to that of the standard glucose concentration, the mutant frequency with limiting glucose was not statistically significant (shown in Table 1, Z-test with $P < 0.05$). These observations suggest that the glucose concentration has a strong impact on IS*1* transposition.

During the stationary phase, the proportion of IS*1* transpositions appeared to be unchanged with limiting and standard glucose concentrations, 18% and 22%, respectively, on the other hand it dropped from 22% to 7% for cultures grown with excess glucose concentration.

IS*1* transposition induced by translation errors

Since at extreme glucose concentrations (limiting or excess) IS*1* transposition was enhanced, we sought to test if this was a result of enhanced translation errors under these physiological conditions. To test this hypothesis streptomycin was provided in growth medium at concentrations that were sub-

Table 3. Distribution of IS*1* transposition at sub-inhibitory concentrations of streptomycin.

Both ZK126 (wt) and GBE 150 (*rpsL150*) strains were grown in M9 and 0.4% glucose unless described in parentheses.

Strain	[Sm][a] μg /ml	Doubling time (min)	Number of mutants analyzed	Insertions (%)			Non insertions (%)
				IS*1*	IS*5*	IS*2*	
Exponential phase							
ZK126	0	90	71	24	3	0	73
ZK126	0.156	90	68	62	3	0	35
ZK126	1.250	90	56	79	0	0	21
GBE150	0	120	59	0	0	0	100
GBE150	0.156	120	61	0	0	2	98
GBE150	1.250	120	59	0	0	2	98
GBE150 (0.1% glucose)	0.156	120	29	10	0	0	90
GBE150 (4% glucose)	0.156	120	36	16	0	0	84
Stationary phase							
ZK126	0	-	69	22	4	0	74
ZK126	0.156	-	23	4	9	0	87
ZK126	1.250	-	28	21	0	0	19
GBE150	0	-	40	5	0	0	95
GBE150	0.156	-	24	4	9	0	87
GBE150	1.250	-	59	3	4	11	82

[a]0.156 and 1.250 μg/ml are respectively 1:32 and 1:4 of MIC. Sm, streptomycin. A Chi-square analysis on the data of ZK126 strain grown in standard glucose containing sub-MIC concentrations of streptomycin indicated that the increase of IS*1* transposition frequency is highly significant at $P < 0.05$.

MIC (i.e., below the minimum inhibitory concentration). At concentrations above the MIC streptomycin blocks protein synthesis, whereas at sub-MIC concentrations the effect is only to reduce translation accuracy without affecting viability (Gorini, 1971; Ruusala & Kurland, 1984). The streptomycin MIC for ZK126 was 5 μg/ml (obtained by double dilution method), and concentrations of 0.156 and 1.25 μg/ml (corresponding to 32 and 4 times lower than the MIC) were used in the fluctuation test analysis. Growth at these streptomycin concentrations had no effect on either culture doubling times or the number of viable cells in the exponential or stationary phase (not shown).

We compared the effect of sub-MIC streptomycin on the transposition of IS*1* into the *bglR* region by growing bacteria with standard glucose concentrations and supplemented with streptomycin (0.156 μg/ml and 1.25 μg/ml) or by growing with limiting or excess glucose concentrations supplemented with 0.156 μg/ml streptomycin (Table 3). When strain ZK126 was grown in a medium with standard glucose without streptomycin the frequency of IS*1* transpositions during exponential growth and stationary phase were comparable (24% and 22%), but transposition was enhanced during exponential growth depending on streptomycin amounts in growth medium, 62% and 79% for 0.156 and 1.25 μg/ml, respectively. A Chi-square analysis on these data indicated that the increase of IS*1* transpositions was highly significant at $P<0.05$. However, the effect of streptomycin was not significant during stationary phase. This may be due to the fact that IS*1* transposition at the *bglR* region was lower (4% with 0.156 μg/ml streptomycin and 21% with 1.25 μg/ml). A Chi-square analysis of the data indicated that IS*1* transpositions were not significantly increased. These results show that IS*1* transposition is greatly increased by streptomycin only during exponential growth, i.e. when the ribosomes are more active, and that limiting or excess glucose concentration could lead to increased translation errors, which in turn is likely to result in an increase of IS*1* transpositions under these physiological conditions.

Translation accuracy and IS*1* transposition

Transposition of IS*1* requires a programmed translational frameshift. The transposase of IS*1* was produced as the InsA-B′-InsB fusion protein by a –1 frameshifting event (Sekine & Ohtsubo, 1989; Escoubas *et al*., 1991). Physical evidence for the synthesis of such InsAB′, composed of domains derived from two consecutive reading frames: *insA* (phase 0) and *insB′* (phase –1) and demonstrated that the InsAB′ protein stimulates IS*1* transposition (Escoubas *et* al., 1994). To examine the role of translation fidelity in IS*1* transposition, strain GBE150 was constructed by replacing the *rpsL* gene of ZK126 strain with the *rpsL150* mutation from strain MC4100 conferring resistance to streptomycin (Materials and Methods). The nucleotide sequencing of the *rpsL* gene from MC4100 and GBE150 strains confirmed that both these strains carried the *rpsL150* mutation as published in (Post & Nomura, 1980), but it differed from the *rpsL* gene of ZK126 by an A→G transition (Genbank accession number AF312716 and AF312717). This mutation resulted in a Lys42 to Arg change. An identical mutation described earlier as *A60* or *sm-5* was shown to result in enhanced translation accuracy (Breckenridge & Gorini, 1970; Funtasu *et al*., 1972). As expected, the *rpsL150* mutation slightly increased the generation time of GBE150 (Levin *et al*., 2000). The mutation rates in GBE150 were reduced by a small fraction when compared to those of strain ZK126 grown under similar conditions. However, the reduction in the mutation rates observed in GBE150 was not statistically significant when examined by the Z-test ($P < 0.05$). The mutation rates reduced in *rpsL150* strain are in agreement with the marginal drop of mutation rates for an *rpsL999* strain documented by Boe (1992).

In contrast to the mutation rates, the presence of the *rpsL150* allele caused a major reduction in IS*1* transposition, it went down from 24% to 0% during the exponential phase for bacteria grown with standard glucose concentrations. This finding may suggest that in the *rpsL150* mutant background translation fidelity is enhanced which in turn is likely to reduce the –1 programmed translation frameshift thus leading to a drop of IS*1* transposition. However, this effect was confined only to exponentially growing bacteria and for unknown reason it disappeared during the stationary phase.

The effect of *rpsL150* on IS*1* transposition was also examined in the exponential phase with sub-MIC streptomycin concentrations, for which the cultures were grown with either limiting or excess glucose concentrations and supplemented with sub-MIC concentration of streptomycin (0.156 μg/ml) and fluctuation analysis was performed. Under these conditions the IS*1* transpositions were restored from 0% (standard glucose) to 10% (0.1% limiting glucose) or 16% (4% excess glucose) (Table 3). These data suggest that the translation fidelity enhanced by *rpsL150* may be insufficient to repress IS*1* transposition in exponential phase bacteria that grew with limiting or excess glucose concentrations.

Transcription analysis of IS*1* transposase

Having shown that IS*1* transposition is induced at sub-MIC concentrations of streptomycin, and at extreme (limited or excess) glucose concentra-

tions, whereas it was repressed by the *rpsL150* mutation, we proceeded to investigate the transcription of the IS*1* transposase gene under these experimental conditions. The functional transposase represents a small subset of peptides that are generated by mistranslation and our aim was to discriminate between an increase in the amount of IS*1* mRNA and an increase of translation infidelities (transcriptional *vs*. post-transcriptional regulation) as alternative mechanisms for the enhanced IS*1* transposition that we observed under conditions of extreme glucose concentrations in the medium and/or at sub-MIC concentrations of streptomycin. Strain ZK126 was grown in M9 with limiting, standard, and excess glucose or in M9 containing standard concentration of glucose supplemented with sub-MIC streptomycin. cDNA was used as a template for conventional RT-PCR amplification and real-time PCR of *insB* and 16S rRNA. Results of conventional RT-PCR and real-time PCR were used to quantify the level of *insB* mRNA, which was expressed as the ratio of the *insB* to 16S-rRNA RT-PCR products (Fig. 3). Compared to strain ZK126 growing in standard glucose concentration without streptomycin, transcription of IS*1* mRNA was lower in all experimental conditions, with the exception of excess glucose where the transcription was enhanced by at least 8-fold. Thus, in most cases, when less IS*1*-mRNA was measured, more IS*1* insertions were observed, suggesting that the regulation of IS*1* transposition occurs principally at a step subsequent to transcription. In the case of excess glucose, the IS*1*-16S ratio increased, suggesting that this condition enhances transcription of (at least) the IS*1* transposase gene or lower 16S transcription. One may then envision that from this larger pool of IS*1* mRNA, a larger amount of misread InsAB transposase may be translated.

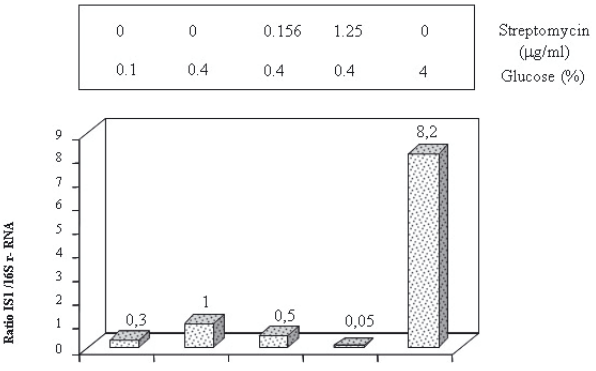

Figure 3. *In vivo* transcriptional analysis of IS*1* by real-time PCR.
Real-time PCR reactions were performed with 50 ng cDNA from cultures grown in M9 supplied with various concentrations of glucose with or without streptomycin. IS*1* transcription was normalized by taking its ratio to 16S rRNA. The normalized ratios of IS*1*/16S rRNA for various conditions are expressed in relation to standard glucose concentration arbitrarily fixed at 1.

DISCUSSION

The present study was aimed at examining the transposition of IS elements in response to manipulations of the host cell physiology by providing different amounts of glucose in the growth medium. Our major finding is that both limiting and excess glucose concentrations enhance transposition of IS*1* into the *bglR* region. The results presented here also demonstrate that IS*1* transposition depends on the translation fidelity of the host cells. This was achieved by provoking translation infidelity by addition of streptomycin at sub-MIC concentrations, and by enhancing translation accuracy by genetic means which in turn inhibited transposition of IS*1* into the *bglR* region. To our knowledge, our findings represent the first experimental evidence demonstrating that limiting and excess glucose or carbon source concentrations enhance translation errors and as a result of this the transposition frequency of an insertion sequence — IS*1* — is increased.

Since transposition of IS*1* requires a programmed translational frameshift to produce a functional transposase InsAB'. Escoubas *et al.* (1994) in their construct that could produce InsAB constitutively without requiring translational frameshift enhanced IS*1* transposition rates by four orders of magnitude confirming that regulation of IS*1* transposition is balance between production of repressor InsA and transposase InsAB'. We assumed that IS*1* transposition is directly controlled by the ability of the cell to promote –1 ribosomal frameshifting which in itself is likely to be dependent on the physiological status of the cells. Indeed, we observed that enhanced (by genetic means) or restricted (by physiology) translation accuracy reduced or increased IS*1* transposition, respectively. Interestingly, the *rpsL150* strain did not allow IS*1* transposition except when glucose was limiting or in excess or when standard glucose cultures were supplied with sub-MIC concentrations of streptomycin. Also in the wild type strain grown at a standard glucose concentration and with streptomycin at sub-MIC, the IS*1* transposition rate was increased in a streptomycin concentration dependent manner. A plausible explanation for these observations would be to suggest that they all depend on ribosomal accuracy, and thus glucose limitation affects IS*1* transposition by increasing the amount of programmed translation frameshifting. In fact, this assumption was further tested by real-time RT-PCR analysis and the results represented here show that *insB* transcription was repressed in all conditions except excess glucose where it was enhanced by 8-fold. However, when compared to standard glucose all the other conditions led to elevated IS*1* transposition into the *bglR* region, which is in agreement with our hypothesis that the regulation of IS*1* transposition in

the studied conditions is primarily at a step subsequent to transcription.

Finding increased IS*1* transposition at extreme glucose is of special interest and may be due to different regulators being involved. Stress-induced accumulation of ppGpp (guanosine tetraphosphate) is known for *E. coli*. In *E. coli* (p)ppGpp synthesis is known to be catalyzed by two paralogous enzymes: synthetase I (*relA* gene product) and synthetase II (encoded by *spoT*), whose activities are regulated differently. Glucose starvation is one of the stressful conditions under which ppGpp is known to accumulate (Hernandez & Bremer, 1991; Xiao *et al.*, 1991; Gentry & Cashel, 1996; for a recent review see: Cashel *et al*., 1996). The glucose starvation conditions described for ppGpp accumulation resemble to those of limiting glucose described in this paper and therefore accumulation of ppGpp in this condition is an interesting possibility to consider. Genetic analysis suggests that SpoT is a bifunctional protein having both ppGpp degradation and synthesis activities (Xiao *et al*., 1991; Hernandez & Bremer, 1991). Accumulation of ppGpp in response to energy source starvation occurs by blocking degradation of ppGpp, catalyzed by a ppGpp 3′-pyrophosphohydrolase encoded by the *spoT* gene. Murray and Bremer (1996) found that both ppGpp synthesis and degradation were decreased immediately after glucose had run out but the rate of degradation was affected more severely leading to accumulation of ppGpp. This observation is interesting and conditions are comparable to that of limiting glucose concentration presented in this article. Interestingly, Gentry and Cashel (1996) also found that the RelA protein activity accounts for the initial burst of ppGpp synthesis at the onset of glucose starvation and that it depends on the presence of amino acids during glucose starvation. Accumulation of ppGpp over a long time period is RelA-independent, and this observation is corroborated by studies in marine *Vibrio* sp. S14 (Flardh *et al*., 1994). In both organisms, *E. coli* and *Vibrio* sp. S14, *relA* mutants are known to exhibit an increase in ppGpp levels but the increase is gradual over a longer period of time. This delay is likely to be due to the time required for expression of some of the genes that are likely be critical for the temporal pattern of ppGpp accumulation suggesting that some carbon-starvation inducible alleles show a *relA* dependence. Since the ZK126 and GBE150 strains used in present study are deleted for *relA*, the accumulation of ppGpp through solitary *spoT* is very likely when grown in limiting glucose. On the other hand, enhanced IS*1* transposition for cultures grown in excess glucose may include yet another regulator(s). Interestingly, in this condition the *cfu* dropped by an order of magnitude between exponential and stationary phase culture. This might suggest that certain deleterious conditions are generated from excess glucose utilization, e.g., accumulation of acids shifting the pH from neutral to acidic. Although both of these conditions exhibited enhanced IS*1* insertions into the *bglR* region, transcription of *insB* was drastically different, reduced by 3-fold for limiting glucose culture while enhanced by 8-fold for excess glucose.

Ribosomal misreading is necessary in *E. coli* because a few essential genes require a programmed translational frameshift (Parker, 1989). Hyper-accurate ribosomes inhibit growth (Ruusala *et al*., 1984). In order to avoid the load of high translation infidelity, translation errors should be kept as low as possible. Indeed, it has been demonstrated that only a small portion from the mRNA pool is mistranslated, often as a result of a programmed translation frameshift sequence (Kurland, 1992). By using a β-galactosidase reporter construct that required a –1 translation frameshift Barak *et al.* (1996) showed that the enzyme activity was enhanced by two orders of magnitude near stationary phase when *E. coli* cells were grown with 0.2% glucose concentration. In an independent study Atkinson *et al*. (1997) showed that glucose-limited cultures exhibited enhanced frameshift frequency by an order of magnitude. The observation described in this paper that IS*1* transposition was enhanced in limiting glucose cultures during exponential phase is in agreement with the above two studies and might represent a similar phenomenon. Also, our results suggest that the biochemical effect of streptomycin on the ribosome mimics conditions that may occur spontaneously in cells starved for amino acids (Atkinson *et al*., 1997).

Interestingly, in nature bacteria are known to grow with a reduced growth rate and with low translation accuracy compared to laboratory strains (Ruusala *et al*., 1984; Kurland, 1992; Mikkola & Kurland, 1992). During nutritional deprivation, IS*1* transpositions are enhanced, and this may play an important role in the process of adaptability of natural populations requiring continuous genome rearrangements to cope with fluctuating environments (Chalmers & Blot, 1999).

We have now identified genetic and physiological tools that enable one to probe the regulation of IS*1* mobility. Nevertheless, the molecular mechanism by means of which either amino acid starvation described in (Atkinson *et al*., 1997) or extreme carbon source studied by Barak *et al*. (1996) and presented in this article influence IS*1* transposition needs to be studied further. In particular, one should focus on the possibility that the growth conditions directly affect ribosome accuracy, or alternatively, that the level of translation fidelity would be under a genetic control.

Acknowledgements

The authors are indebted to B. Michel and J. Adams, B.G. Hall and D. Thaler for their helpful criticisms, and thank M.S. Fox, W. Arber, R. d'Ari, and D. Schneider for valuable discussions. We are grateful to A. Tomasz for valuable discussion and critics. The authors are grateful to a reviewer for the suggestions that helped to clarify the subject and improve the manuscript.

REFERENCES

Atkinson J, Dodge M, Gallant J (1997) Secondary structures and starvation-induced frameshifting. *Mol Microbiol* **26**: 747–753.

Barak Z, Gallant J, Linsdley D, Kwieciszewki B, Heidel D (1996) Enhanced ribosome frameshifting in stationary phase cells. *J Mol Biol* **263:** 140–148.

Boe L (1992) Translational errors as the cause of mutations in *Escherichia coli*. *Mol Gen Genet* **231:** 469–471.

Breckenridge L, Gorini L (1970) Genetic analysis of streptomycin resistance in *E. coli*. *Genetics* **65:** 9–25.

Bull HJ, McKenzie GJ, Hastings PJ, Rosenberg SM (2000) Evidence that stationary-phase hypermutation in the *Escherichia coli* chromosome is promoted by recombination. *Genetics* **154:** 1427–1437.

Casadaban MJ (1976) Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J Mol Biol* **104:** 541–555.

Cashel M, Gentry DR, Hernandez VJ, Vinella D (1996) The stringent response. In *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology* (Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE, eds), pp 1458–1496. American Society of Microbiology, Washington DC.

Chalmers R, Blot M (1999) Insertion sequences and transposons In *Organisation of the Procaryotic Genome* (Charlebois RL ed) pp 151–169. ASM Press, NY.

Chao L, McBroom SM (1985) Evolution of transposable elements: an IS10 insertion increases fitness in *Escherichia coli*. *Mol Biol Evol* **2:** 359–369.

Chao L, Vargas C, Spear BB, Cox EC (1983) Transposable elements as mutator genes in evolution. *Nature* **303**: 633–635.

Cooper VS, Schneider D, Blot M, Lenski RE (2001) Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B. *J Bacteriol* **183**: 2834–2841.

Defez R, De Felice M (1981) Cryptic operon for β-glucoside metabolism in *Escherichia coli* K12: genetic evidence for a regulatory protein. *Genetics* **97:** 11–25.

Di Nardo S, Voelkel KA, Sternglanz R, Reynolds AE, Wright A (1982) *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. *Cell* **31:** 43–51.

Escoubas JM, Prère MF, Fayet O, Salvignol I, Galas D, Zerbib D, Chandler M (1991) Translational control of transposition activity of the bacterial insertion sequence IS*1*. *EMBO J* **10**: 705–712.

Escoubas JM, Lane D, Chandler M (1994) Is the IS1 transposase, InsAB', the only IS1-encoded protein required for efficient transposition? *J Bacteriol* **176**: 5864–5867.

Flardh K, Axeberg T, Albertson NH, Kjelleberg S (1994) Stringent control during carbon starvation of marine *Vibrio* sp. strain S14: molecular cloning, nucleotide sequence, and deletion of the *relA* gene. *J Bacteriol* **176**: 5949–5957.

Funtasu G, Nierhaus K, Wittman HG (1972) Determination of allele types and amino-acid exchanges in protein S12 of 3 streptomycin-resistant mutants of *E. coli*. *Biochim Biophys Acta* **287:** 282–291.

Gentry DR, Cashel M (1996) Mutational analysis of the *Escherichia coli spoT* gene identifies distinct but overlapping regions involved in ppGpp synthesis and degradation. *Mol Microbiol* **19**: 1373–1384.

Giel M, Desnoyer M, Lopilato J **(**1996) A mutation in a new gene, *bglJ*, activate the *bgl* operon in *Escherichia coli*-K12. *Genetics* **143:** 627–635.

Gorini L (1971) Streptomycin and misreading of the genetic code. In *Ribosomes* (Nomura M, Tissièress A, Lengyel P, eds) pp 791–803. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hall BG (1998) Activation of the *bgl* operon by adaptive mutation. *Mol Biol Evol* **15**: 1–5.

Hall BG (1999a) Spectra of spontaneous growth-dependent and adaptive mutations at *ebgR*. *J Bacteriol* **181**: 1149–1155.

Hall BG (1999b) Transposable elements as activators of cryptic genes in *E. coli*. *Genetica* **107**: 181–187.

Hernandez VJ, Bremer H (1991) *Escherichia coli* ppGpp synthetase II activity requires *spoT*. *J Biol Chem* **266**: 5991–5999.

Kurland CG (1992) Translational accuracy and the fitness of bacteria. *Ann Rev Genet* **26:** 29–50.

Lea DE, Coulson CA (1949) The distribution of the numbers of mutants in bacterial populations. *J Genet* **49:** 264–285.

Levin BR, Perrot V, Walker N (2000) Compensatory mutations, antibiotic resistance and the population genetics of adaptative evolution in bacteria. *Genetics* **154:** 985–997.

Mahillon J, Chandler M (1998) Insertion sequences. *Microbiol Mol Biol Rev* **62:** 725–774.

Mikkola R, Kurland CG (1992) Selection of laboratory wild-type phenotype from natural isolates of *Escherichia coli* in chemostats. *Mol Biol Evol* **9**: 394–402.

Moorthy S, Mahadevan S (2002) Differential spectrum of mutations that activate the *Escherichia coli bgl* operon in an *rpoS* genetic background. *J Bacteriol* **184**: 4033–4038.

Murray DK, Bremer H (1996) Control of *spoT*-dependent ppGpp synthesis and degradation in *Escherichia coli*. *J Biol Chem* **259**: 41–57.

Orgel LE, Crick FH (1980) Selfish DNA: the ultimate parasite. *Nature* **284**: 604–607.

Parker J (1989) Errors and alternatives in reading the universal genetic code. *Microbiol Rev* **53:** 273–298.

Post LE, Nomura M (1980) DNA sequences from the *str* operon of *Escherichia coli*. *J Biol Chem* **255:** 4660–4666.

Reynolds AE, Felton J, Wright A (1981) Insertion of DNA activates the cryptic *bgl* operon in *E. coli* K12. *Nature* **293**: 625–629.

Rodriguez H, Snow ET, Bhat U, Loechler EL (1992) An *Escherichia coli* plasmid-based, mutational system in which *supF* mutants are selectable — insertion elements dominate the spontaneous spectra. *Mutat Res* **270:** 219–231.

Ruusala T, Kurland CG (1984) Streptomycin preferentially perturbs ribosomal proofreading. *Mol Gen Genet* **198:** 100–104.

Ruusala T, Andersson D, Ehrenberg M, Kurland CG (1984) Hyper-accurate ribosomes inhibit growth. *EMBO J* **3:** 2575–2580.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press.

Schneider D, Faure D, Noirclerc-Savoye M, Coursange E, Blot M (2000) A broad-host range plasmid to isolate mobile genetic elements in gram negative bacteria. *Plasmid* **44:** 201–207.

Schneider D, Duperchy E, Coursange E, Lenski RE, Blot M (2001) Long-term experimental evolution in *Escherichia coli*. IX. Characterization of insertion sequence-mediated mutations and rearrangements. *Genetics* **156**: 477–488.

Schnetz K, Rak B (1992) IS5 — a mobile enhancer of transcription in *Escherichia coli*. *Proc Natl Acad Sci USA* **89**: 1244–1248.

Schnetz K, Toloczyki C, Rak B (1987) β-Glucosides (*bgl*) operon of *Escherichia coli* K-12: nucleotide sequence, genetic organization, and possible evolutionary relationship to regulatory components to two *Bacillus subtilis* genes. *J Bacteriol* **169:** 2579–2590.

Sekine Y, Ohtsubo E (1989) Frameshifting is required for production of the transposase encoded by insertion sequence 1. *Proc Natl Acad Sci USA* **86:** 4609–4613.

Shiga Y, Sekine Y, Kano Y, Ohtsubo E (2001) Involvement of H-NS in transpositional recombination mediated by IS1. *J Bacteriol* **183:** 2476–2484.

Treves DS, Manning S, Adams J (1998) Repeated evolution of an acetate-crossfeeding polymorphism in long-term populations of *Escherichia coli*. *Mol Biol Evol* **15**: 789–797.

Ueguchi C, Ohta T, Set C, Suzuku T, Mizuno T (1998) The *leuO* gene product has a latent ability to relieve bgl silencing in *E. coli*. *J Bacteriol* **180:** 190–193.

Xiao H, Kalman M, Ikehara K, Zemel S, Glaser G, Cashel M (1991) Residual guanosine 3′,5′-bispyrophosphate synthetic activity of *relA* null mutants can be eliminated by *SpoT* null mutations. *J Biol Chem* **266**: 5980–5990.