RESEARCH NOTE

Accumulation of *hns* mutations specifically in stationary phase in an *E. coli* strain carrying an impaired *rpoS* locus

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Introduction

Interplay between regulators in a cell can lead to differential gene regulation and allow the organism to respond to a range of stimuli. In bacteria, this is exemplified by the global regulators RpoS and H-NS. RpoS, the σ^s subunit of RNA polymerase, acts as a master regulator of a subset of stationary-phase-specific genes (for a review see Loewen and Hengge-Aronis 1994). The genes under the *rpoS* regulon are specifically induced when cells encounter stressful conditions for growth such as nutrient deprivation experienced during stationary phase. H-NS is an abundant nucleoid structuring protein, which has been classified as a global transcriptional silencer. H-NS preferentially binds to DNA that has an intrinsic curvature and represses many genes that are required to tide over environmental stress (Goransson *et al*. 1990; Owen-Hughes *et al*. 1992). H-NS also regulates RpoS at the posttranscriptional level (Yamashino *et al*. 1995). The opposing actions of these two global regulators in the bacterial cell help in the homeostatic control of the genes of the *rpoS* regulon. Many *hns* strains also carry spontaneously arising second-site mutations in the *rpoS* gene (Barth *et al*. 1995). These may be selected to prevent the constitutive expression of the genes of the *rpoS* regulon that may be detrimental to the cell. This is consistent with the report that *hns rpoS* double mutants have a faster doubling time than *rpoS* single mutants (Barth *et al*. 1995).

The *bgl* operon, encoding the functions necessary for the uptake and catabolism of β-glucosides, is silent and uninducible in wild-type *E. coli* and is activated by spontaneous *cis*-acting and *trans*-acting mutations (Mukerji and Mahadevan 1997, and references therein). Mutations in the *hns* locus constitute a major class among the *trans*-acting mutations that activate the operon. Earlier work in our laboratory had shown that the profile of activating mutations differs between *rpoS*$^+$ and *rpoS* strains of *E. coli*, with the *rpoS* strain showing a higher proportion of *hns* mutations (Moorthy and Mahadevan 2002). The reasons for this difference remained unknown. One likely possibility is that there is a selection for *hns* mutations in strains that do not express a functional RpoS. This may allow the cells to enhance the basal level of expression of the genes of the *rpoS* regulon that are negatively regulated by *hns*, offering an advantage specifically in the stationary phase. In such an event, the enhanced occurrence of *hns* mutations is likely to be seen in the stationary phase and not during exponential phase of growth where the mutations do not provide any specific advantage.

Activation of the silent *bgl* operon was used to monitor the frequency of mutations that accumulate in the *hns* locus in *E. coli* strains that carry either the wild-type *rpoS* allele or a disruption of the *rpoS* locus. The frequency of *hns* mutations remained low in the wild-type strain under all growth phases. However, a higher frequency of *hns* mutations was seen in a strain carrying a disruption of the *rpoS* locus, specifically in the stationary phase, suggesting positive selection for such mutations in the stationary phase when *rpoS* function is compromised. Implications of these results in terms of the functioning of the two global regulators are discussed.

Materials and methods

Strains and plasmids

The strains and plasmids used in this study are listed in table 1.

Transformation

Transformation of the plasmid pHMG409 was carried out by $CaCl_2$ method as described previously (Sambrook *et al*. 1989).

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Transduction

P1 transductions to determine linkage to *hns* were carried out as described previously (Miller 1992).

Results

Isolation of the Bgl$^+$ mutants

The spectrum of Bgl$^+$ mutations obtained in the stationary phase was compared to that in the exponential phase in the two *E. coli* strains SM2 (*rpoS*::Tn*10*) and MM1 (*tnaA*::Tn*10*) that are otherwise isogenic (table 1). Exponential-phase cultures of MM1 and SM2 were plated on minimal salicin plates and incubated at 37°C. The Bgl$^+$ mutants obtained at the end of 48 h of incubation are expected to be predominantly spontaneous mutants that preexisted in the exponential-phase culture used for plating. The same plates on further incubation (96 h) gave rise to additional Bgl$^+$ mutants that emerge post-plating, i.e. when the cells are in the stationary phase. Fifty Bgl$^+$ mutants each from the exponential phase as well as stationary phase obtained from five independent sets of plating of the two strains were subjected to further analysis.

Profile of the Bgl$^+$ mutants

To detect mutations in *hns*, the Bgl$^+$ mutants were transformed with the plasmid pHMG409 carrying the wild-type *hns* gene. Loss of the Bgl$^+$ phenotype in the presence of the plasmid indicated the presence of a mutation in the *hns* locus that is now rescued by the wild-type allele carried on the plasmid. Alternatively, using the strain MG1655Tn5 that carries a Tn*5* transposon linked to the wild-type *hns* locus as the donor, P1 transductions were performed. Loss of Bgl$^+$ phenotype in 80% of the Kan^R transductants was taken as an indication of linkage of the mutation to the *hns* locus. The results are shown in table 2 as the fraction of *hns*-linked Bgl$^+$ mutants (the ratios of hns-linked Bgl$^+$ mutants over the total number of Bgl$^+$ mutants). The fraction of *hns*-linked stationary-phase Bgl$^+$ mutants is significantly higher ($P < 0.0082$) in the *rpoS* strain SM2. The mutation profile is not changed in the case of MM1 (*rpoS*$^+$) with approximately equal proportions of *hns* mutations in both exponential and stationary phases.

Comparing the Bgl$^+$ mutation profile in another control strain

These results suggest a positive selection for *hns* mutations when *rpoS* function is compromised and occurs specifically in the stationary phase. The strains used were isogenic and differed only at the *rpoS* and *tnaA* loci that carried the transposon Tn*10*. No interaction between the *tnaA* locus and *hns* is known and therefore the loss of *tnaA* function in MM1 is unlikely to influence the results. However, since *tnaA* gene is linked to the *bgl* operon and is positively regulated by RpoS (Lacour and Landini 2004) we made use of an additional *rpoS*$^+$ control strain that had a Tn*10* insertion in an unrelated locus.

Table 1. Bacterial strains and plasmids used in this study.

Strain/plasmid	Description	Reference or source
MM1	RV (F′ Δ*lacX74 thi bglR*^{0}) *tnaA* :: Tn*10* [Bgl$^-$]	M. Mukerji
SM2	RV *rpoS*::Tn*10* [Bgl$^-$]	Moorthy and Mahadevan 2002
MG1655 Tn5	MG 1655 Tn5 (80% linked to *hns*$^+$) [*Kan*^{R}]	J. Gowrishankar
RV Tn10	RV *srlA*::Tn*10* [Bgl$^-$]	This work
ZK819	ZK126 (W3110 Δ*lacU169*) *rpoS819* Sm^R [Bgl$^-$]	Zambrano *et al*. 1993
pHMG409	*Eco*RI–*Stu*I *hns*$^+$ fragment in pLG339	Goransson *et al*. 1990

Table 2. Fraction of *hns*-linked Bgl$^+$ mutants of SM2 and MM1.

No.	Exponential phase		Stationary phase	
	SM2 (*rpoS*)	MM1 (*rpoS*$^+$)	SM2 (*rpoS*)	MM1 (*rpoS*$^+$)
1	0.2	0.2	0.8	0.1
2	0.0	0.0	0.7	0.1
3	0.0	0.2	0.4	0.2
4	0.3	0.2	0.6	0.3
5	0.0	0.2	0.6	0.1

The fraction of *hns*-linked Bgl$^+$ mutants is expressed as the ratio of *hns*-linked mutants over the total number of Bgl$^+$ mutants. The mean of the fraction of *hns*-linked stationary-phase Bgl$^+$ mutants of SM2 (0.62) was significantly different from the respective mean of MM1 stationary-phase Bgl$^+$ mutants (0.18) as analysed using the Mann–Whitney *U* test ($P < 0.0082$).

Twenty exponential and stationary-phase Bgl$^+$ mutants of RV (*srlA*::Tn*10*) obtained from four independent isolations were analysed similarly. There was no difference in the fraction of *hns*-linked exponential and stationary-phase Bgl$^+$ mutants and the profile was similar to that of MM1.

Bgl$^+$ mutation profile in an rpoS-attenuated strain

Prolonged starvation of cells can lead to the selection of *rpoS* mutations that confer a growth advantage in the stationary phase or GASP (Zambrano *et al*. 1993). These mutants show partial or attenuated expression of *rpoS*. Subsequent incubation could promote selection of *hns* mutations that confer an additional growth advantage. If this hypothesis is true, it follows that the late-arising Bgl$^+$ mutants of MM1 (*rpoS*$^+$) will also show enhanced fraction of *hns*-linked Bgl$^+$ mutants in the stationary phase as they are likely to have accumulated *rpoS* mutations that confer a GASP phenotype upon prolonged incubation. Thirty independently isolated late-arising Bgl$^+$ mutants of MM1, obtained 5, 6 and 7 days post-plating on minimal salicin medium, were subjected to analysis. There was no significant difference in the fraction of *hns*-linked exponential-phase Bgl$^+$ mutants and late-arising Bgl$^+$ mutants of MM1 while late-arising Bgl$^+$ mutants of SM2 continued to show increased *hns* linkage. This could be because prolonged starvation leads to selection of specifically attenuated *rpoS* alleles that show reduced activity and not null mutations in *rpoS* (Zambrano *et al*. 1993), which may not lead to positive selection of *hns* mutations. To test this, 20 exponential and stationary-phase Bgl$^+$ mutants from two independent isolations of an *rpoS*-attenuated strain ZK819 were analysed similarly. The spectrum of exponential and stationary-phase Bgl$^+$ mutants did not differ with respect to their *hns* linkage in ZK819.

Discussion

The silent *bgl* operon of wild-type *E. coli* is predominantly activated by mutations in the regulatory region *bglR*. However, in an *rpoS* strain, *hns* mutations are more predominant among the mutations that activate the operon. The mutually antagonistic action of RpoS and H-NS for regulation of the *rpoS* regulon genes could lead to this difference. RpoS is essential for the expression of the *rpoS* regulon genes only in the stationary phase while H-NS inhibits the expression of some of these genes. Therefore, *hns* mutations might be selected in an *rpoS* strain specifically in the stationary phase as they compensate for the loss of the positive control by RpoS on these genes. To test this, activation of the silent *bgl* operon was studied in the exponential and stationary phase of *E. coli* strains carrying a wild-type or a null allele of *rpoS*. The results reported here suggest a positive selection for *hns* mutations when *rpoS* function is compromised specifically in the stationary phase. Moreover, the nature of the *rpoS* allele (attenuated versus null) is significant in the subsequent selection of *hns* mutations. The *bgl* operon was used only as a marker to follow the occurrence of *hns* mutations and is unlikely to contribute to the skewed distribution of mutations. Though *hns* negatively regulates the operon and mutations in the *hns* locus activate the operon, *cis*-acting mutations such as transposition and insertion elements within the regulatory locus *bglR* also help overcome negative regulation by *hns*. These do occur predominantly in the *rpoS*$^+$ background and during the exponential phase in the *rpoS* background. Therefore, the occurrence of *hns* mutations in the *rpoS* background specifically in the stationary phase is unlikely to be an effect influenced by the *bgl* locus. However, the results do not specify the exact growth advantage offered by the loss of *hns* activity in an *rpoS* background. The advantage may be the result of elevated expression of multiple functions homeostatically controlled by the two global regulators. Our results underscore the fact that stains carrying mutations in global regulators are likely to accumulate secondary mutations under specific selective conditions that can alter their presumed genotype.

Acknowledgements

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