

Natalia Pasternak Taschner · Ezra Yagil  
Beny Spira

## The effect of IHF on $\sigma^S$ selectivity of the *phoA* and *pst* promoters of *Escherichia coli*

Received: 23 August 2005 / Revised: 13 December 2005 / Accepted: 20 December 2005 / Published online: 11 January 2006  
© Springer-Verlag 2006

**Abstract** The *pst* operon, a member of the PHO regulon of *Escherichia coli*, encodes a high-affinity phosphate transport system whose expression is induced when the cells enter a phase of phosphate starvation. The expression of *pst* is stimulated by the integration host factor (IHF). Transcription of the PHO regulon genes is initiated by the RNA polymerase complexed with  $\sigma^D$  ( $E\sigma^D$ ). Owing to a cytosine residue at position  $-13$  of the *pst* promoter its transcription can also be initiated by  $E\sigma^S$ . Here, we show that inactivation of IHF in vivo abolishes the  $\sigma^S$ -dependent transcription initiation of the *pst* operon, indicating that both  $-13C$  residue and IHF are required to confer on *pst* the ability to be transcribed by  $E\sigma^S$ . Introduction of a  $-13C$  residue in the promoter region of *phoA*, another PHO regulon gene that is not directly affected by IHF, did not affect its exclusive transcription initiation by  $E\sigma^D$ .

**Keywords** PHO regulon · *pst* operon · IHF · *rpoS* · *Escherichia coli*

### Introduction

*Escherichia coli* possesses several architectural proteins that mimic the function of histones by assisting DNA folding and compaction. One of the best-characterised histone-like proteins is the integration host factor (IHF), which is a relatively abundant small DNA-bending

protein (Goosen and van de Putte 1995). IHF is an asymmetric protein that binds DNA at specific sites, causing a bent of  $160^\circ$  in the DNA molecule (Rice et al. 1996). It is a heterodimer composed of subunits IhfA and IhfB that are encoded by *ihfA* and *ihfB*, formerly known as *himA* and *himD* or *hip* (Weisberg et al. 1996).

Integration host factor has first been characterized as an accessory protein in the site-specific recombination process of phage  $\lambda$  (Miller and Friedman 1980), but it is also involved in the modulation of bacterial gene expression (Goosen and van de Putte 1995). The level of IHF increases in the stationary growth phase (Ditto et al. 1994) and several stress-specific  $\sigma^S$ -dependent promoters, such as *ihfA*, *dps*, *osmY* and *sra*, are known to be regulated by IHF (Arfin et al. 2000; Lange et al. 1993; Izutsu et al. 2001). In some cases, the presence of an IHF binding site in the promoter region favours transcription by the  $\sigma^S$  factor, while in others transcription is inhibited by IHF (Aviv et al. 1994; Colland et al. 2000).

The PHO regulon of *E. coli* consists of more than 40 genes and operons whose transcription is induced by inorganic phosphate (Pi) starvation. Among them are *phoA*, which encodes alkaline phosphatase (AP) and the *pst* operon, that encodes the high affinity Pi uptake system Pst. Pst also possesses a negative regulatory function, as most mutations in the *pst* operon impose constitutivity on all PHO genes (Wanner 1996). Despite being stress-dependent, transcription of the PHO genes is initiated by RNA polymerase complexed with  $\sigma^D$  ( $E\sigma^D$ ; Makino et al. 1996). The only exception is *pst*, which, in addition to its transcription by  $E\sigma^D$ , can also be transcribed by  $E\sigma^S$  (Taschner et al. 2004). A reason for that is probably the unique presence of a cytosine residue in the  $-13$  position at the *pst* promoter region, a feature shared by most  $\sigma^S$  dependent promoters (Lacour et al. 2003; Lee and Gralla 2001; Weber et al. 2005). It has also been demonstrated that the *pst* promoter region possesses a functional IHF binding site that is located between the *pst* promoter and the Shine-Dalgarno sequence, and that IHF stimulates *pst* transcription (Spira and Yagil 1999).

N. P. Taschner · B. Spira (✉)  
Departamento de Microbiologia,  
Instituto de Ciências Biomédicas,  
Universidade de São Paulo,  
Av. Prof. Lineu Prestes 1374,  
CEP 05508-900 São Paulo, Brazil  
E-mail: benys@usp.br  
Tel.: 55-11-30917347  
Fax: 55-11-30917354

E. Yagil  
Department of Biochemistry, Tel-Aviv University,  
69978 Tel-Aviv, Israel

In the present report we show that IHF plays a positive role in the  $E\sigma^S$ -dependent transcription of the *pst* operon and that a  $-13T$  to  $-13C$  mutation in the IHF-independent promoter of *phoA* does not permit its transcription by  $E\sigma^S$ .

## Methods

### Strains and plasmids

These are listed in Table 1.

### Growth media and growth conditions

Medium A is a semi-rich medium that is low in Pi (Levinthal et al. 1962). In the high-Pi medium it is supplemented with 1 mM  $KH_2PO_4$ . For the assays of AP and chloramphenicol acetyl transferase (CAT), cells were grown overnight in low-Pi medium A.

### Enzyme assays

Alkaline phosphatase was assayed using *p*-nitrophenyl-phosphate (*p*-NPP) as substrate as described (Spira et al. 1995). AP activity is represented by the increase in absorbance at 410 nm  $min^{-1}$  per OD unit at 540 nm. CAT assays were performed essentially as described (Taschner et al. 2004) using 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) together with acetyl-CoA and chloramphenicol as substrates. The increase rate in absorbance at 412 nm was recorded and CAT activity was calculated as nmoles  $min^{-1}$  (mg protein) $^{-1}$ .

### Plasmid construction

Plasmid pNP4 was constructed by amplifying the *phoA* fragment from the chromosome of strain MG1655 using the oligonucleotides CAGCATTCTGCAGACGAT

AC and ATTTTCATAGCAGGATCCCTCTTCA (bold letters indicate the restriction sites for PstI and BamHI introduced by point modifications). The *phoA* PCR fragment was then digested with BamHI and PstI and ligated to the same sites of plasmid pUC19. Plasmid pNP8 was constructed by digesting plasmid pNP4 with BamHI and *Hind*III and ligating the resulting *phoA* fragment to the same sites of the low-copy plasmid pGB2.

### Site-directed mutagenesis and DNA sequencing

Site-directed mutagenesis was performed by the circular mutagenesis method, using double stranded DNA template and selection with DpnI, as described (Sambrook and Russel 2001). Plasmid pNP8, carrying the intact *phoA* gene, was used as template for the PCR reaction. The oligonucleotides CTGTCATAAAGTTGTCACG GCCGAGACCTATAGTCGCTTTG and CAAAGCG ACTATAGG TCTCGGCCGTGACAACCTTTATGAC AG carried the desired mutation (underlined). The amplification product was treated with DpnI and transformed into strains JY16 and NP23. Both wild type and mutated plasmids were sequenced in an automatic sequencer type ABI Prism 3100 Genetic Analyser (Applied Biosystems/Hitachi, Warrington, UK) to confirm the presence of the point mutation.

## Results and discussion

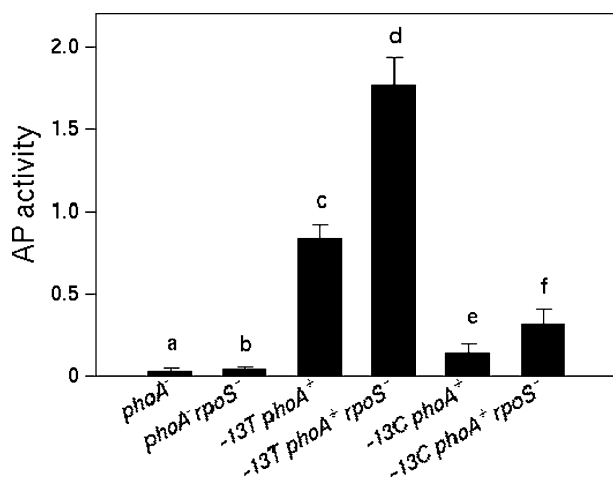
In a previous report we have demonstrated that replacing the cytosine residue at position  $-13$  of the *pst* promoter with a thymine abolishes its ability to be transcribed by  $E\sigma^S$  (Taschner et al. 2004), indicating that the  $-13C$  residue is important for promoter recognition by  $\sigma^S$ . Since the *pst* promoter is the only PHO promoter that can be recognized by  $\sigma^S$  and is the only one that displays the consensus binding site of  $\sigma^S$  ( $-13$ -CTANNNT) (Lee and Gralla 2001; Weber et al. 2005), we tested if this  $\sigma^S$   $-10$  consensus site is sufficient to

**Table 1** Strains and plasmids used in this study

| Strains and plasmids | Genotype                                                                    | Source                       |
|----------------------|-----------------------------------------------------------------------------|------------------------------|
| Bacteria             |                                                                             |                              |
| JY16                 | <i>phoA</i>                                                                 | (Rao et al. 1993)            |
| NP23                 | JY16 <i>rpoS::Tn10</i>                                                      | This study                   |
| NP69                 | RW1370 <i>rpoS::Tn10</i>                                                    | This study                   |
| NP108                | RW1369 <i>rpoS::Tn10</i>                                                    | This study                   |
| RW1369               | F <sup>-</sup> <i>thyA strA</i>                                             | (Weisberg et al. 1996)       |
| RW1370               | F <sup>-</sup> <i>thyA strA ihfB (hip157)</i>                               | (Weisberg et al. 1996)       |
| Plasmids             |                                                                             |                              |
| pBS11                | <i>pstS-cat</i> transcriptional fusion                                      | (Taschner et al. 2004)       |
| pGB2                 | Cloning vector                                                              | (Churchward et al. 1984)     |
| pNP4                 | <i>phoA</i> <sup>+</sup> cloned in pUC19                                    | Lab collection               |
| pNP8                 | <i>phoA</i> <sup>+</sup> cloned in pGB2                                     | This study                   |
| pNP9                 | <i>phoA</i> (carrying a $-13T \rightarrow C$ point mutation) cloned in pGB2 | This study                   |
| pUC19                | Cloning vector                                                              | (Yanisch-Perron et al. 1985) |

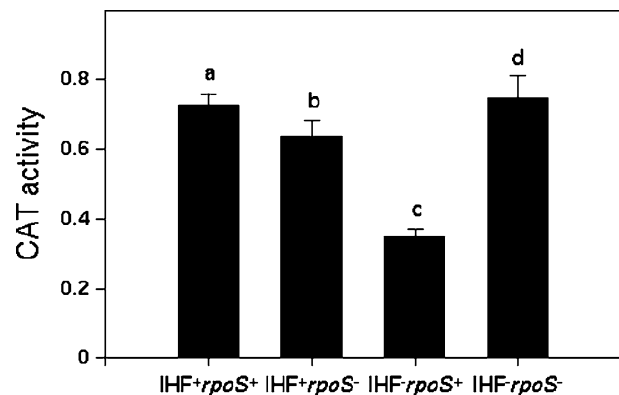
allow transcription of other PHO genes by  $E\sigma^S$ . The  $-10$  region of the *phoA* promoter ( $-13$ -TTATAGT) differs from the  $\sigma^S$  consensus in that it carries a  $-13$  thymine residue. The  $-13$  thymine of the *phoA* promoter was therefore replaced with a cytosine residue (plasmid pNP9) to test if *phoA* would now be able to be transcribed by  $E\sigma^S$ . Plasmid pNP8 carries the wild-type promoter. Two isogenic *phoA*-mutant strains, one with and the other without an *rpoS* knockout mutation, were transformed with each of these plasmids and were assayed for AP activity under conditions of Pi-starvation (Fig. 1). The untransformed *rpoS*<sup>+</sup> and *rpoS*<sup>-</sup> control strains (Fig. 1, columns a, b) show only a negligible AP activity. The assay of these two host strains transformed with pNP8 that expresses *phoA* under the wild-type promoter (columns c, d) confirms that the presence of  $\sigma^S$  partially represses AP activity (Taschner et al. 2004). When the two host strains were transformed with pNP9 that expresses *phoA* under the  $-13C$  promoter mutation (columns e, f) the expression of AP was strongly diminished, but the presence of  $\sigma^S$  still inhibited AP activity, i.e., unlike the *pst* promoter, the  $\sigma^S$  consensus of the *phoA* promoter, provided by the  $-13T \rightarrow C$  substitution does not support any  $E\sigma^S$ -mediated transcription of *phoA*. This suggests that another factor is required by  $E\sigma^S$  to initiate transcription from a *phoA* promoter. This extra factor could be the presence of a functional IHF binding site, which is present in the control region of *pst* (Spira and Yagil 1999).

To test if IHF is involved in the  $\sigma^S$ -promoted transcription initiation of the *pst* operon, a strain carrying a mutation in the *ihfB* locus and its isogenic *ihf*<sup>+</sup> parent, each with and without an *rpoS* knockout mutation, were transformed with plasmid pBS11 that carries a truncated



**Fig. 1** Effect of *rpoS* on AP activity of bacteria carrying a  $-13T \rightarrow C$  mutation in the *phoA* promoter. Cells were grown overnight in medium A and assayed for AP. **a** *phoA*-negative mutant JY16; **b** *phoA* *rpoS* double mutant NP23; **c** plasmid pNP8 (*pphoA*<sup>+</sup>) in strain JY16; **d** plasmid pNP8 in strain NP23; **e** plasmid pNP9 (*phoA*<sup>+</sup> carrying a  $-13T \rightarrow C$  mutation) in strain JY16; **f** plasmid pNP9 in strain NP23. Bars represent the means  $\pm$  SD of three independent experiments

*pst* operon fused to the promoterless *cat* gene. Thus, the product of the *cat* gene, CAT, served as a reporter for the expression of the *pst* promoter. The transformants were grown overnight under limited Pi concentration and assayed for CAT activity (Fig. 2). In an IHF<sup>+</sup> background, the activity of CAT was mildly inhibited by the *rpoS* mutation (Fig. 2; columns a, b), confirming that  $\sigma^S$  contributes to the overall transcription level of *pst* (Taschner et al. 2004). However, in the absence of IHF, *pst* expression was significantly inhibited by  $\sigma^S$  (Fig. 2, columns c, d), suggesting that IHF plays a positive role in the  $\sigma^S$ -transcription initiation of *pst*. In other words, in the absence of IHF  $E\sigma^S$  has lost its ability to compete with  $E\sigma^D$  in recognizing the *pst* promoter causing it to behave like *phoA* and the other PHO genes, which are inhibited by the presence of  $\sigma^S$  (Taschner et al. 2004). Moreover, the positive effect of IHF on overall *pst* transcription (compare columns a, c in Fig. 2) is abolished in the absence of a functional  $\sigma^S$  (compare columns b, d). These data indicate that the combination of the  $-10$   $\sigma^S$  consensus and the presence of IHF are important for *pst* transcription by  $E\sigma^S$ . Though the  $-10$  region of *pst* (CTATTTT) follows the consensus of a  $\sigma^S$ -dependent promoter (Lee and Gralla 2001; Weber et al. 2005), in vivo it still requires IHF in order to be transcribed by  $E\sigma^S$ . Furthermore, the *phoA* promoter bearing the  $-13T \rightarrow C$  mutation does carry the  $\sigma^S$   $-10$  consensus (CTATAGT), nevertheless it is still unable to be transcribed by  $E\sigma^S$ . A direct effect of *rpoS* on *ihfB* expression and vice-versa cannot be completely ruled out, but it is unlikely. This is because the expression of *ihfB* is only slightly affected by  $\sigma^S$  (Aviv et al. 1994) and because  $\sigma^S$  is mostly post-transcriptionally regulated in starved cells (Zgurskaya et al. 1997). It should be mentioned that the promoter region of *phoA* also carries a putative IHF-binding site that deviates from the IHF-binding site consensus by two



**Fig. 2** Effect of IHF and of *rpoS* on the expression of the *pst* operon. Cells transformed with plasmid pBS11 (*pst-cat* fusion) were grown overnight in medium A and assayed for CAT activity. **a** plasmid pBS11 in strain RW1369 (*ihf*<sup>+</sup> *rpoS*<sup>+</sup>); **b** plasmid pBS11 in strain NP108 (*ihf*<sup>+</sup> *rpoS*<sup>-</sup>); **c** plasmid pBS11 in strain RW1370 (*ihfB* *rpoS*<sup>+</sup>); **d** plasmid pBS11 in strain NP69 (*ihfB* *rpoS*<sup>-</sup>). Bars represent the means  $\pm$  SD of three independent experiments

base pairs, but an in vitro analysis showed that it is not functional (Spira and Yagil 1999).

Four other genes (*osmY*, *sra*, *ihfA* and *dps*) have hitherto been reported to be regulated by both  $\sigma^S$  and IHF (Izutsu et al. 2001; Aviv et al. 1994; Colland et al. 2000; Altuvia et al. 1994). *dps* and *sra* are positively regulated, while *osmY* and *ihfA* are negatively affected by IHF. The *pst* operon can be added to the list of genes and operons that are positively regulated by  $\sigma^S$  and IHF.

In conclusion, although *pst* is predominantly transcribed by  $E\sigma^D$ , we suggest that the interaction between IHF,  $E\sigma^S$  and the *pst* promoter with its  $-13C$  residue, enables *pst* to be transcribed also by  $E\sigma^S$ . As suggested before (Taschner et al. 2004), the elevated transcription level of *pst* by  $E\sigma^S$  inhibits the expression of the other PHO genes (that are transcribed by  $E\sigma^D$  alone) and consequently provides free RNA polymerase core units to interact with  $\sigma^S$ . This fine-tuning is responsible for the trade-off between the expression of genes related to survival and expression of genes related to growth.

## References

- Altuvia S, Almiron M, Huisman G, Kolter R, Storz G (1994) The *dps* promoter is activated by OxyR during growth and by IHF and sigma S in stationary phase. *Mol Microbiol* 13:265–272
- Arfin SM, et al. (2000) Global gene expression profiling in *Escherichia coli* K12. The effects of integration host factor. *J Biol Chem* 275:29672–29684
- Aviv M, Giladi H, Schreiber G, Oppenheim AB, Glaser G (1994) Expression of the genes coding for the *Escherichia coli* integration host factor are controlled by growth phase, *rpoS*, ppGpp and by autoregulation. *Mol Microbiol* 14:1021–1031
- Churchward G, Belin D, Nagamine Y (1984) A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. *Gene* 31:165–171
- Colland F, Barth M, Hengge-Aronis R, Kolb A (2000) Sigma factor selectivity of *Escherichia coli* RNA polymerase: role for CRP, IHF and lrp transcription factors. *EMBO J* 19:3028–3037
- Ditto MD, Roberts D, Weisberg RA (1994) Growth phase variation of integration host factor level in *Escherichia coli*. *J Bacteriol* 176:3738–3748
- Goosen N, van de Putte P (1995) The regulation of transcription initiation by integration host factor. *Mol Microbiol* 16:1–7
- Izutsu K, et al (2001) *Escherichia coli* ribosome-associated protein SRA, whose copy number increases during stationary phase. *J Bacteriol* 183:2765–2773
- Lacour S, Kolb A, Landini P (2003) Nucleotides from  $-16$  to  $-12$  determine specific promoter recognition by bacterial sigma S-RNA polymerase. *J Biol Chem* 278:37160–37168
- Lange R, Barth M, Hengge-Aronis R (1993) Complex transcriptional control of the sigma S-dependent stationary-phase-induced and osmotically regulated *osmY* (*csi-5*) gene suggests novel roles for Lrp, cyclic AMP (cAMP) receptor protein-cAMP complex, and integration host factor in the stationary-phase response of *Escherichia coli*. *J Bacteriol* 175:7910–7917
- Lee SJ, Gralla JD (2001) Sigma38 (*rpoS*) RNA polymerase promoter engagement via  $-10$  region nucleotides. *J Biol Chem* 276:30064–30071
- Levinthal C, Signer ER, Fetherolf K (1962) Reactivation and hybridization of reduced alkaline phosphatase. *Proc Natl Acad Sci USA* 48:1230–1237
- Makino K, Amemura M, Kawamoto T, Kimura S, Shinagawa H, Nakata A, Suzuki M (1996) DNA binding of PhoB and its interaction with RNA polymerase. *J Mol Biol* 259:15–26
- Miller HI, Friedman DI (1980) An *E. coli* gene product required for lambda site-specific recombination. *Cell* 20:711–719
- Rao NN, Roberts MF, Torriani A, Yashphe J (1993) Effect of *glpT* and *glpD* mutations on expression of the *phoA* gene in *Escherichia coli*. *J Bacteriol* 175:74–79
- Rice PA, Yang S, Mizuuchi K, Nash HA (1996) Crystal structure of an IHF-DNA complex: a protein-induced DNA U-turn. *Cell* 87:1295–1306
- Sambrook J, Russel D (2001) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Spira B, Silberstein N, Yagil E (1995) Guanosine 3',5'-bisphosphate (ppGpp) synthesis in cells of *Escherichia coli* starved for Pi. *J Bacteriol* 177:4053–4058
- Spira B, Yagil E (1999) The integration host factor (IHF) affects the expression of the phosphate-binding protein and of alkaline phosphatase in *Escherichia coli*. *Curr Microbiol* 38:80–85
- Taschner NP, Yagil E, Spira B (2004) A differential effect of sigma S on the expression of the PHO regulon genes of *Escherichia coli*. *Microbiology* 150:2985–2992
- Wanner B (1996) *Escherichia coli* and *Salmonella*: cellular and molecular biology. In: Neidhardt FC, Curtiss RI (eds) *ASM Press*, Washington DC, pp 1357–1381
- Weber H, Polen T, Heuveling J, Wendisch VF, Hengge R (2005) Genome-wide analysis of the general stress response network in *Escherichia coli*: sigma S-dependent genes, promoters, and sigma factor selectivity. *J Bacteriol* 187:1591–1603
- Weisberg RA, et al (1996) Nomenclature of the genes encoding IHF. *Mol Microbiol* 19:642
- Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 33:103–119
- Zgurskaya HI, Keyhan M, Matin A (1997) The sigma S level in starving *Escherichia coli* cells increases solely as a result of its increased stability, despite decreased synthesis. *Mol Microbiol* 24:643–651