IHF is a trans-acting factor implicated in the regulation of the proU P2 promoter

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One-Sentence Summary: IHF is a new regulator of the proU promoter involved in the fine tuning of its expression.

Editor: Andre Klier

ABSTRACT

IHF is a protein of the bacterial nucleoid proteins (NAPs for nucleoid-associated proteins) involved in DNA structuring and transcription regulation. In vivo interplay between different NAPs determines selectively the expression rate of many genes. Here, we show that IHF is a trans-acting factor implicated directly in the regulation of the proU promoter of Escherichia coli by binding specifically and solely around the promoter box. proU expression is mainly under the repression effect of another NAP, H-NS. We show that IHF binding to proU organize the promoter DNA local structure in a completely different way than H-NS binding. Thus, we propose that the partial alleviation of H-NS repression is mediated by the promoter structure modification.

Key words: osmoregulation; IHF; NAPs; H-NS; proU

INTRODUCTION

An important challenge with which bacteria must cope concerns fluctuations in external osmolarity. Despite the existence of aquaporins in many microorganisms (Tanghe, van Dijck and Thevelein 2006), it is worth recalling that no bacterial cell can actively pump water across the semi-permeable cytoplasmic membrane to compensate for the water influxes or effluxes raised by changes in the external osmolarity (Wood 2011). As a consequence, microorganisms have to balance the vital osmotic gradient indirectly by influencing the osmotic potential of the cytoplasm to direct the flux of water in or out of the cell. They accumulate water-attracting ions and organic osmolytes when they face hyperosmotic conditions to prevent cellular dehydration (Kempf and Bremer 1998), and they expel these compounds upon osmotic downshift (Hoffmann et al., 2008; Haswell, Phillips and Rees 2011). A direct and effective response to osmotic stress could increase the fitness of pathogen bacteria in harsh environments, and would be essential for their viability and virulence mechanism. The Salmonella Enteritidis strain is the best-illustrated example, as among other virulence genes, the ones related to osmotic stress are expressed more in the S. Enteritidis strain described as high pathogenic in comparison to the low pathogenic serovar (Shah et al., 2013).

The cellular stress response to high osmolarity is a multiphasic process (Bremer and Krämer 2000) starting with the rapid uptake of K+ ions (Whatmore, Chudek and Reed 1990) and the subsequent replacement of this ion with a class of organic osmolytes that are highly compliant with cellular physiology (Kempf and Bremer 1998). While effective water management is certainly the cornerstone for the cellular response to high salinity by most microorganisms (Wood 2011), the overall
adjustment process to this environmental challenge is a rather complex process implicating many different effectors, some of the best known amongst them being products of the proU operon.

The proU operon encodes for a transport system that mediates the accumulation of compatible solutes such as glycine-betaine, L-proline and related compounds during cell growth in media with elevated osmolarity. It has been shown to be highly regulated by the salinity and superhelicity (Gowrishankar and Manna 1996). Recently, osmoregulation of proU on the post-transcriptional level was described, involving RNaseIII that processes proU RNA in a specific manner. This processing is efficient and it imposes upon the DNA structure (Lang et al., 1997). The proposed model of repression of proU by H-NS suggests a strong influence of RNA in a specific manner. This processing is efficient and it imposes upon the DNA structure (Lang et al., 1997).

Fragments containing the proU promoter region extending from −68 to +303, with +1 referring to the transcription start site of the proU P2 promoter were obtained by PCR using a combination of one unlabeled primer and a second primer end-labeled with [γ-32P] ATP (3000 Ci mmol–1) using phage T4 polynucleotide kinase and 5′-FAM-GTCTGGCCATTGAAG-3′. The fluorescently marked DNA fragments obtained by the primer extension were run on a capillary electrophoresis sequencer (ABI 3500 capillary sequencer—Applied Biosystems) alongside standard size markers (LIZ 600 size standards—Applied Biosystems). The data obtained were normalized against the size standard from Applied Biosystems and analyzed with the software Grams 32. The scans were normalized against a non-varying peak as an arbitrary signal in order to compare the different amplitudes and therefore quantities of each DNA fragment. A log of the ratio of the normalized scans of the himA(−) or the hns(−) to the ut was calculated and represented.

**RESULTS AND DISCUSSION**

**IHFs bind specifically to the proU P2 promoter**

Analysis of the proU promoter region via Virtual Footprint (Hales et al., 1994) predicted two binding sites for IHF to the promoter.
region at positions −25/−11 and +134/+149 relative to the +1 site of the P2 promoter. On the coding strand, IHF specifically protected a region extending from position −33 to +25 from DNase I indicated by a black bar in Fig. 1. The initial protection site of IHF is very specific and can be distinguished at nM range concentrations (see lane d and e) from −12 to +20. Several hyperactive sites at position −5, +30, +51 and +110 were also observed. Such hypersensitivity to DNase I indicates bending of the whole promoter region, a characteristic of IHF interaction (Rice et al., 1996). Above concentrations of 250 nM of IHF, a general protection of the fragment is observed indicating non-specific binding of the protein to the DNA. Thus, in vitro IHF is able to bind to the proU promoter in a specific manner in a nanomolar affinity range. Interestingly, no specific footprints were observed outside of the core promoter region (−30, +30) as shown on the resolved region of the gel for the non-coding strand. However, at higher concentrations non-specific binding was observed. These results showed that IHF in an nM concentration range binds specifically near the transcription start site on the proU promoter. Conversely in this region surrounding the +1, H-NS binds with a very low affinity (Bouffartigues et al., 2007) suggesting that IHF could easily compete out H-NS for binding at this RNAP binding region.

IHF is implicated in the regulation of the proU P2 promoter in vivo

Since we observed IHF binding in vitro, we then looked at the activity of the promoter in vivo in the absence of IHF. The strain E. coli W3110 wt and E. coli W3110 himA(−) carrying the pKKproV-GFP plasmid were grown in medium at high or low salt concentrations and the activity of the promoter was followed by measuring GFP fluorescence. proV-GFP promoter expression occurs during the mid-exponential growth phase in both the wt and the himA(−) strain. The rate of change in fluorescence intensity is plotted as a function of time (Fig. 2) comparing the difference in these values for wt and himA(−) at the point of maximum change in rate for the wt showed that IHF is able to induce 4-fold activation on this promoter in a low salt background. However, in the presence of high salt (LB supplemented with 0.3 M NaCl) there is already a high proU expression (Gowrishankar and Manna 1996), and so there is no measurable difference between the wt and the himA(−) strain (data not shown) on the expression of the proU promoter suggesting that IHF is acting only when expression is down regulated by H-NS, in this case at low salt concentrations. Thus, we propose that IHF helps to relieve H-NS repression of the proU promoter in an off state rather than by acting as an activation factor. To explain the regulation of proU promoter, models in the literature proposed either that osmotic regulation
is mediated by changes in DNA supercoiling without involving trans-acting factors (Higgins et al., 1988) or suggested a model where proU expression is mediated by potassium glutamate instead of DNA supercoiling (Ramirez et al., 1989; Prince and Vilclarejo 1990). Additionally, it has been shown that H-NS represses the expression of the proU P2 promoter at low osmolarity by binding to the NRE (Dattananda, Rajkumari and Gowrishankar 1991; Rajkumari et al., 1996). Here, we did not observe any effect of IHF on proU expression at high osmolarity suggesting that IHF did not mediate osmoregulation of the proU promoter.

The increase up to 4-fold in proU-P2 promoter expression in the presence of IHF occurs at low salt concentrations where the promoter is under H-NS repression showing that IHF was partially alleviating H-NS repression. Since these two nucleoid proteins have been reported to have a direct effect on DNA topology (Dorman 2009), we therefore decided to check their effect on the local structure of the promoter.

**Effect of IHF and H-NS on the local structure of the proU promoter DNA region**

Characterization of the in vivo interaction of IHF with its specific binding site on a given promoter using UV laser photoprotection has been previously reported (Valls et al., 2002). This study showed that UV laser footprints for IHF are similar in vivo and in vitro.

The property of UV light to induce photoreactions in the DNA is very sensitive to the immediate environment of the bases (Pemberton et al., 2002). Binding of IHF and bending the DNA results in a re-orientation of the planes of consecutive bases thus increasing or decreasing the probability of an intramolecular photoreaction such as the formation of a pyrimidine dimer. Thus, following dimer formation provides an excellent tool to assess the local structure reflecting the DNA topology of a promoter bound by its regulatory proteins.

We used in vivo laser photoprotection to compare the local conformation of proU promoters in the presence or absence of IHF or H-NS. The intensities of bands appearing due to termination before pyrimidine dimers is an indication of the probability of their formation and a comparison of signals in wt and mutant himA(−) (where there is H-NS but not IHF) or hns(−) (with IHF but no H-NS) is shown in Fig. 3; for comparison the ratio of these intensities is shown as a log_2 plot. As can be seen in Fig. 3, the absence of IHF in vivo had an effect on the structure of DNA around the P2 transcription start site (+1). The probability of thymidine formation increased significantly in the absence of IHF in comparison to the wt, while in the absence of H-NS the opposite effect was observed (Fig. 3). This means that these two proteins topologically organize the promoter region and that under these conditions IHF moderates the strong modification observed when only H-NS is present (see profile of the himA(−) strain, Fig 3). These changes in vivo in the fine DNA structure of the promoter
at the P2 proU transcription start site imposed by H-NS and IHF suggest a model where the proteins instead of preventing or helping transcription by steric impeachment modify the local structure of the nucleic acid offering a substrate for the polymerase that is optimized or not for initiation of transcription depending on the environmental conditions. The DNA base pairs affected by the modification in the local structure of the double helix either by the effect of H-NS or IHF are indicated by the gray box in Fig. 4. We also schematically display the H-NS and IHF binding sites on the proU promoter. We thus show that IHF is implicated in relieving H-NS repression at least at low osmolality by a mechanism implying a control of the promoter local structure. Other examples exist where IHF and H-NS interplay to modulate gene expression: in the hflA promoter of the S. enterica, IHF is required to alleviate H-NS-mediated silencing depending upon growth conditions (Queiroz et al., 2011); P. van Ulsen and N. Goosen have reported that, in vivo, in E. coli the extent of IHF-mediated Fe promoter activation differs in the presence of H-NS (van Ulsen et al., 1997). Furthermore, in Vibrio cholerae the tcpA promoter Stonehouse et al. proposed that IHF might function at this promoter to alleviate H-NS repression (Stonehouse et al., 2008). Therefore, high osmolality is not the only factor relieving H-NS repression from the proU promoter and osmolarity, DNA supercoiling, H-NS and IHF are all actors involved in the fine-tuning of proU expression.

ACKNOWLEDGEMENTS

The authors thank F Boccard for providing them the ut and the himA(–) strain. The ANR Grant DAMAGE (ANR-09-BLAN-0367) supported this work. They are grateful to Malcolm Buckle for his fruitful comments and discussions.

FUNDING

AK was supported by a doctoral fellowship from the French Ministry of Research.

Conflict of interest statement. None declared.

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