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RESEARCH LETTER

Expression of the *Escherichia coli ompW* colicin S4 receptor gene is regulated by temperature and modulated by the H-NS and StpA nucleoid-associated proteins

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Abstract

The OmpW family consists of a ubiquitous group of small outer membrane (OM) β-barrel proteins of Gram-negative bacteria with proposed roles in environmental adaptation but poorly understood mechanisms of expression. We report here that *Escherichia coli* K-12 OmpW contents are drastically modified by temperature changes compatible with the leap from the environment to warm-blooded hosts and/or vice versa. Thus, while OmpW is present in the OM of bacteria grown at 37 °C, it sharply disappears at 23 °C with the concomitant acquisition of colicin S4 resistance by the cells. *ompW::lacZY* fusions indicated that temperature regulation operates at the level of transcription, being *ompW* expression almost abolished at 23 °C as compared to 37 °C. Moreover, *E. coli* Δ*hns* mutants lacking H-NS showed reductions in *ompW* transcription and OmpW contents at 37 °C, indicating positive modulatory roles for this nucleoid-structuring protein in *ompW* expression. Also, Δ*hns*Δ*stpA* double mutants simultaneously lacking H-NS and its paralog StpA showed more severe reductions in *ompW* expression at 37 °C, resulting in the complete loss of OmpW. The overall results indicate that OmpW contents in *E. coli* are regulated by both temperature and H-NS and reinforce OmpW functions in bacterial adaptation to warm-blooded hosts.

Introduction

The regulation of the production of outer membrane (OM) proteins of Gram-negative bacteria in response to different environmental cues plays a pivotal role in the adaptation of these organisms to changing environments (Lin *et al.*, 2002; Nikaido, 2003; De la Cruz & Calva, 2010; McClean, 2012). OmpW, an OM protein that forms part of the colicin S4 receptor in *Escherichia coli* (Pilsl *et al.*, 1999; Arnold *et al.*, 2009), is a member of a widespread family of so-called small β-barrel proteins composed by 210–230 amino acid residues structured in 8 transmembrane β-strands and extended loops exposed to the environment (Nikaido, 2003; De la Cruz & Calva, 2010; McClean, 2012). The contents of OmpW have been found to vary significantly in response to different environmental cues both in *E. coli* and in many other Gram-negative bacterial species, thus suggesting functions in environmental adaptation (Bina *et al.*, 2003; Hu *et al.*, 2005; Nandi *et al.*, 2005; Xu *et al.*, 2006; Gil *et al.*, 2007; Lin *et al.*, 2008, 2010; Wu *et al.*, 2009; McClean, 2012). Most notably, some environmental stimuli increase OmpW contents while others reduce it (Bina *et al.*, 2003; Hu *et al.*, 2005; Nandi *et al.*, 2005; Xu *et al.*, 2006; Gil *et al.*, 2007; Lin *et al.*, 2008, 2010; Wu *et al.*, 2009), thus suggesting the involvement of a complex regulatory network in this process whose details are largely unknown.

Temperature change is a well-known cue used by both commensalistic and pathogenic bacteria signaling the transfer from the external environment into warm-blooded hosts and the necessity to adjust gene expression accordingly (De la Cruz & Calva, 2010; Shapiro & Cowen, 2012). In this work, we demonstrate that *E. coli* OmpW contents are regulated by temperature changes compatible with the leap from environmental sources to potential hosts and vice versa. We also found that *ompW*

expression was significantly reduced in *E. coli* Δ*hns* mutant cells bearing deletions in the *hns* gene encoding the histone-like nucleoid-structuring H-NS protein (Fang & Rimsky, 2008; Stoebel *et al.*, 2008; Dillon & Dorman, 2010; Shapiro & Cowen, 2012). Moreover, *ompW* expression impairments were exacerbated in Δ*hns*Δ*stpA* double mutants additionally lacking StpA, an H-NS paralog and proposed molecular backup. As these nucleoid-binding proteins impose both local and large scale-constraints on DNA topology and their binding activities are sensitive to temperature variations (Fang & Rimsky, 2008; Stoebel *et al.*, 2008; Dillon & Dorman, 2010; Shapiro & Cowen, 2012), the overall results provide evidence for complementary roles of H-NS and StpA in favoring a particular DNA architecture enhancing productive *ompW* expression at temperatures compatible with potential warm-blooded hosts.

Materials and methods

Bacterial strains and culture conditions

All bacterial strains were grown aerobically in LB liquid or solid medium containing the appropriate antibiotics when corresponding (Km 30 μg mL^{-1}; chloramphenicol 15 μg mL^{-1}) at 23 or 37 °C as indicated in the legends to figures. Solid LB medium included 1.5% (w/v) Bacto agar. The *E. coli* K-12 strain BW25113 (*lacI*^{q} *rrnBT*14 Δ*lacZ*WJ16 *hsdR*514 Δ*araBADAH33* Δ*rhaBADLD78*) and the isogenic Δ*ompW*, Δ*hns*, and Δ*stpA* single mutants (in which the corresponding genes were replaced by a *npt* cassette conferring kanamycin (Km) resistance) were obtained from the Keio collection (Baba *et al.*, 2006). To construct the Δ*hns*Δ*stpA* double mutant of this strain, the *npt* cassette was first removed from the Δ*hns::npt* mutant by transforming cells with the temperature-sensitive plasmid pCP20 carrying the FLP recombinase (Cherepanov & Wackernagel, 1995). The resulting kanamycin-sensitive single mutants (Δ*hns**) were then used as hosts for P1 transduction using lysates obtained from Δ*stpA::npt* bacteria (Miller, 1972) to generate Δ*hns**Δ*stpA::npt* double mutants. A similar procedure was employed to remove the *npt* cassette from these cells to generate kanamycin-sensitive Δ*hns**Δ*stpA** double mutants.

The *E. coli* K-12 strain MC4100 [F^{-}, *araD139*, Δ*(argF-lac)169*, λ^{-}, *e14*$^{-}$, *flhD5301*, Δ*(fruK-yeiR)725(fruA25)*, *relA1*, *rpsL150*(str^{R}), *rbsR22*, Δ*(fimB-fimE)632(::IS1)*, *deoC1*] was from the laboratory stock.

Colicin S4 sensitivity assay

The *E. coli* 5K strain bearing plasmid pHP189 (Pilsl *et al.*, 1999) was the source of colicin S4. After growing overnight in LB liquid medium at 37 °C, the cells were collected by centrifugation (15 000 ***g*** for 10 min), resuspended in 50 mM Tris-HCl (pH 7.5), glycerol 10% (v/v), and subjected to sonic disruption. Crude extracts were then cleared by centrifugation as above, and the supernatant was used for colicin sensitivity assays.

To test colicin S4 sensitivity, the BW25113 (wt) strain was grown overnight in LB liquid medium at 23 or 37 °C. Aliquots of 0.1 mL of this culture were overlaid on LB agar plates previously kept at 23 or 37 °C, and 5 μL of 10-fold serial dilutions of colicin S4 crude extracts prepared as above were then dropped into the agar surface following a clockwise pattern as shown in Fig. 1a. The plates were further incubated for 24–48 h at the corresponding temperature, and the differences in colicin sensitivity were recorded. A BW25113 Δ*ompW* mutant was also included in the assay as a control of colicin S4 resistance (Pilsl *et al.*, 1999; Arnold *et al.*, 2009).

Analysis of OM proteins by SDS–polyacrylamide gel electrophoresis

Escherichia coli OM fractions were isolated following previously described protocols (Brambilla *et al.*, 2014) and analyzed by SDS-PAGE analysis using 12% (w/v) polyacrylamide gels (Sambrook *et al.*, 1989). The relative levels of OmpW as compared to OmpA in the OM of the different bacterial strains at 23 or 37 °C were estimated by densitometric scanning of the gels with the aid of ImageQuant, version 5.2 (Molecular Dynamics, Inc.). The OmpA protein was selected for this purpose because *ompA* expression is not subjected to temperature regulation (White-Ziegler & Davis, 2009).

Construction of *lacZY* transcriptional fusions

The *npt* cassette was removed from Δ*ompW::npt* or Δ*yciC::npt* mutants of the Keio collection (Baba *et al.*, 2006) as described earlier. The scar sequence left behind containing the 34 nucleotide FRT site was used to generate the corresponding *lacZY* transcriptional fusions (Ellermeier *et al.*, 2002). All constructions were verified using PCR using the corresponding forward primers (Baba *et al.*, 2006) and oligonucleotide 5′-GTAACCGACC CAGCGCCC-3′ as the reverse primer annealing 567-bp downstream of the *lacZ* initiation codon (Ellermeier *et al.*, 2002). To construct Δ*hns*, Δ*stpA*, or Δ*hns*Δ*stpA* mutants containing *ompW::lacZY* fusions, the *npt* cassettes were first removed from the corresponding mutants as described above, and the resulting kanamycin-sensitive cells were then used as hosts for P1 transduction using lysates obtained from BW25113 *ompW::lacZY* bacteria

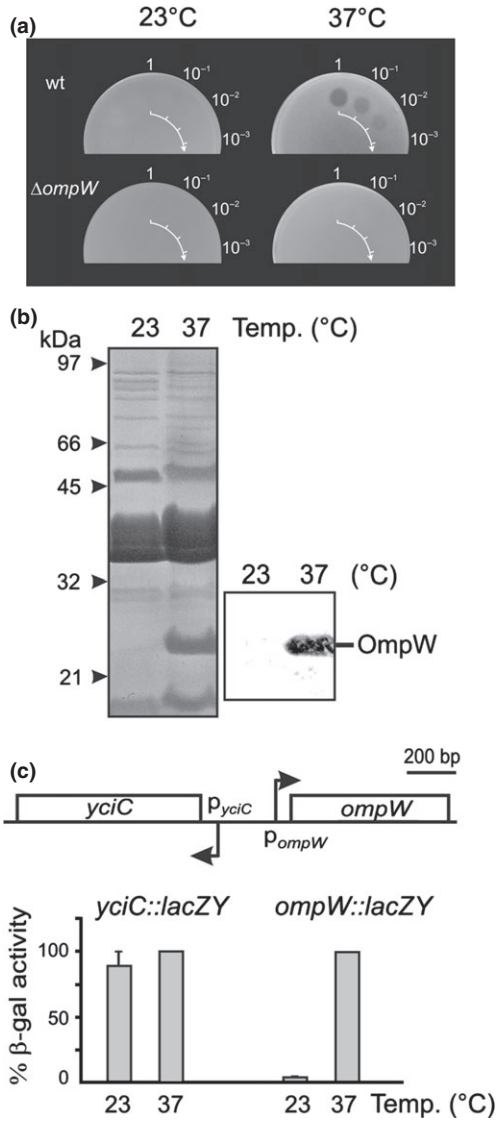

Fig. 1. Temperature regulation of *Escherichia coli* OmpW contents. (a) Colicin S4 sensitivity assay of BW25113 (wt) grown at 23 or 37 °C. Five μL of 10-fold serial dilutions of colicin S4 crude extracts were dropped into the agar surface following a clockwise pattern as indicated. Isogenic Δ*ompW* mutants (Δ*ompW*) are also included as controls of complete colicin resistance. (b) Left, SDS–polyacrylamide gel electrophoresis analysis of OM fractions isolated from BW25113 cells grown in LB medium at 23 or 37 °C. Right, immunoblot analysis of the corresponding OM fractions using anti-OmpW. The equivalent to 30 μg of total OM proteins was loaded on each well in all cases. (c) Expression of *ompW::lacZY* transcriptional fusions in BW25113 cells grown at 23 or 37 °C. The schematic representation of the *E. coli* genomic locus containing the divergently encoded *ompW* and *yciC* genes and the corresponding transcription initiation sites (Mendoza-Vargas *et al.*, 2009; Zhou & Rudd, 2013) are shown at the top. β-galactosidase values for a given fusion indicate the percentage with respect to the value measured at 37 °C, and the mean and standard deviation of at least three independent measurements are shown. For details, see Materials and methods.

constructed as described above following conventional procedures (Miller, 1972).

β-galactosidase activities were determined at 30 °C in chloroform-permeabilized cells using ortho-nitrophenyl-β-galactoside (ONPG) as substrate following described protocols (Miller, 1972). Aliquots of bacterial cultures grown in LB medium at 23 or 37 °C to an $A_{600\ nm}$ of 2.5 were employed for this purpose, thus reflecting *ompW* expression over long-term growth. The β-galactosidase activities in each case were first calculated in Miller units, and the results expressed as the percentage of the units obtained for each strain as compared to the activity measured for the wt strain at 37 °C.

OmpW purification, antibody production, and immunoblot analysis

The OmpW protein used as antigen was purified from OM fractions of *E. coli* BL21 (DE3) cells containing plasmid pHP211 (Pilsl *et al.*, 1999) by preparative SDS-PAGE (Sambrook *et al.*, 1989) using 12% polyacrylamide gels. Polyclonal antibodies were elicited in rabbits following conventional procedures (Sambrook *et al.*, 1989). Immunoblot analysis was performed by transferring the proteins from the SDS-PAGE gels to polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia) followed by OmpW detection with rabbit polyclonal antibodies as first antibody and alkaline phosphatase-conjugated anti-rabbit IgG as second antibody as described (Brambilla *et al.*, 2014).

Results and discussion

Escherichia coli OmpW contents are regulated by temperature

In the course of our studies on the biogenesis of *E. coli* OmpW, we observed that *E. coli* BW25113 (wt) cells showed markedly increased resistance to colicin S4 when grown at 23 °C as compared to 37 °C (Fig. 1a). As OmpW forms part of the *E. coli* colicin S4 receptor and bacterial mutants lacking this OM protein (Δ*ompW* mutants) show full resistance to this colicin (Pilsl *et al.*, 1999; Arnold *et al.*, 2009) as also shown in Fig. 1a, the above observations suggested that OmpW contents were strongly reduced or even absent at 23 °C. To analyze this possibility, we first compared the OmpW contents in the OM of *E. coli* BW25113 cells grown to stationary phase at 23 and 37 °C (Fig. 1b). As seen in the figure, both SDS-PAGE and immunoblot analyses indicated almost nondetectable amounts of OmpW in the OM of cells grown at 23 °C, thus explaining the colicin resistance of cells grown at this temperature. The same results were

obtained with the *E. coli* K-12 strain MC4100 (not shown).

ompW expression is regulated by temperature at the transcription level

The drastic reduction in *E. coli* OmpW contents observed at 23 °C could reflect temperature regulation of *ompW* transcription or, alternatively, different post-transcriptional processes at the level of protein secretion or transit to the OM (Lin *et al.*, 2002; Nikaido, 2003; De la Cruz & Calva, 2010; McClean, 2012). We thus constructed *ompW::lacZY* transcriptional fusions on BW25113 cells to analyze the effects of growth temperature on *ompW* expression. Expression of the *yciC* gene located in the same genomic locus than *ompW* but divergently oriented (Fig. 1c) was also analyzed using *yciC::lacZY* fusions, to study whether this particular gene clustering bears regulatory relevance. As seen in Fig. 1c, very low *ompW* transcription was detected at 23 °C as compared to 37 °C, as judged by the levels of β-galactosidase activity obtained in cells bearing *ompW::lacZY* fusions collected at late exponential phase. Similar differences were also obtained in cells grown at 23 or 37 °C to early exponential phase ($A_{600\ nm}$ = 1.0, data not shown).

The overall results thus reinforce the notion that temperature regulation of *ompW* expression resides mainly at the transcriptional level. On the contrary, no significant differences in the expression of the *yciC* gene were observed between 23 and 37 °C (Fig. 1c), thus indicating that temperature regulation is restricted to the *ompW* gene in this common genomic locus.

Genetic identification of the H-NS and StpA nucleoid-associated proteins as modulators of *ompW* expression

The nucleoid-structuring H-NS protein is an abundant DNA-binding protein involved in the regulation of a substantial number of temperature-responsive *E. coli* genes (White-Ziegler & Davis, 2009). We thus decided to analyze whether H-NS could also mediate temperature regulation of *ompW* expression, and for this purpose, we measured both *ompW* expression and OM OmpW contents in *E. coli* Δ*hns* mutants grown at 23 and 37 °C (Fig. 2). As seen in Fig. 2a, *ompW* transcription was negatively affected at 37 °C by H-NS loss as judged by the almost 50% reduction in the relative levels of *ompW::lacZY* expression found in Δ*hns* mutants when compared to wt cells grown at this temperature (Fig. 2a). The *ompW* transcript levels attained at 37 °C in Δ*hns* mutants, however, were still sufficient to induce significant synthesis of OmpW as judged by the relative content of this protein in the OM of these mutants which were around 60% of the wt strain (Fig. 2b and c). At 23 °C, *ompW* transcription in Δ*hns* mutants was highly impaired similarly to the case of wt cells (Fig. 2a). This reduced *ompW* transcriptional activity was also negatively affected by H-NS loss as judged by further reductions in β-galactosidase activity observed in Δ*hns* mutants as compared to wt cells at 23 °C (Fig. 2a). Thus, the overall results shown above suggest separable effects of temperature and H-NS on *ompW* expression.

Escherichia coli contains an H-NS paralog, StpA, which shares around 58% identity with H-NS and also belongs to the nucleoid-associated proteins group (Sonden & Uhlin, 1996; Zhang *et al.*, 1996; Stoebel *et al.*, 2008; Dillon & Dorman, 2010). Although *stpA* deletion does not promote a notable phenotype to *E. coli* as compared to *hns* deletion, the current evidence suggests both complementary as well as individual roles for these two proteins on gene expression and also in thermoregulation (Sonden & Uhlin, 1996; Zhang *et al.*, 1996; Johansson *et al.*, 1998; Dillon & Dorman, 2010).

We thus analyzed next whether StpA could play complementary roles to H-NS in mediating *ompW* expression, and for this purpose, we analyzed *ompW::lacZY* expression and OmpW contents in Δ*stpA* and Δ*hns*Δ*stpA* mutants and compared these results with those of Δ*hns* single mutants discussed earlier (Fig. 2). As seen in Fig. 2, *ompW* expression at 37 °C was impaired in Δ*hns*Δ*stpA* cells to an extent even larger than that observed in Δ*hns* single mutants, as judged by both β-galactosidase activity values (Fig. 2a) and the absence of OmpW in the corresponding OM fractions (Fig. 2b and c). On the contrary, no impairment on *ompW::lacZY* expression at 37 °C was observed in Δ*stpA* single mutants, and actually, relative increases of β-galactosidase activity values were obtained in these cells (Fig. 2a). Still, this increased *ompW* transcriptional activity observed in Δ*stpA* mutants was not reflected in higher OmpW contents as judged by the similar relative amounts of this OM protein between Δ*stpA* mutants and wt cells (Fig. 2b and c). At 23 °C, *ompW* transcription was highly impaired in both Δ*stpA* and Δ*hns*Δ*stpA* mutants, similarly to the case of wt and Δ*hns* mutants shown above (Fig. 2a).

The observations above thus indicated positive roles for H-NS in the regulation of *E. coli ompW* expression. Moreover, they also pointed for complementary roles between H-NS and StpA in mediating *ompW* expression, thus reinforcing the idea that StpA can function as a molecular backup for the activating role of H-NS (Sonden & Uhlin, 1996; Zhang *et al.*, 1996; Johansson *et al.*, 1998). However, the observation that *ompW* expression was significantly affected in Δ*hns* but not in Δ*stpA* single mutants (Fig. 2) also disclosed differences

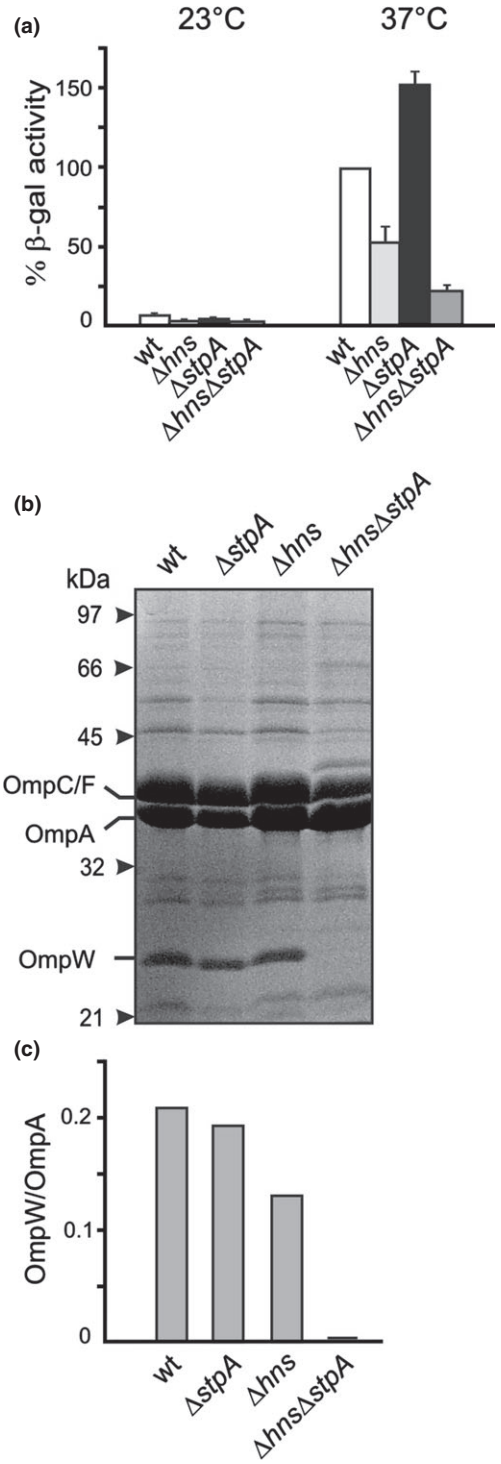

Fig. 2. Positive modulation of *Escherichia coli ompW* expression by H-NS. (a) Expression of *ompW* in BW25113 (wt), Δ*hns*, Δ*stpA*, and Δ*hns*Δ*stpA* cells grown at 23 or 37 °C. β-galactosidase values indicate the percentage with respect to 37 °C, and the mean and standard deviation of at least three independent measurements are shown. (b) SDS-PAGE analysis of OM fractions derived from the above cells grown at 37 °C. The final positions of OmpW as well as the OmpC/OmpF and OmpA porins (Brambilla *et al.*, 2014) are shown on the left margin. The final positions of the molecular mass markers are also indicated. (c) Relative OmpW contents (as compared to OmpA) in the OM fractions of cells shown in (b). For details, see Materials and methods.

between H-NS and StpA in mediating this regulation. The higher *ompW* transcription levels found in Δ*stpA* cells at 37 °C (Fig. 2) could be explained by the loss of the StpA repressive activity on *hns* expression (Zhang *et al.*, 1996). The higher H-NS contents expected on these cells could result in consequent stimulations of *ompW* expression as suggested from the results of this work.

H-NS is an abundant bacterial protein whose binding to the chromosome imposes both local and large scale-constraints on DNA topology which may affect gene expression, usually in a negative manner (Fang & Rimsky, 2008; Stoebel *et al.*, 2008; Dillon & Dorman, 2010; Shapiro & Cowen, 2012). Moreover, H-NS binding to DNA seems extremely sensitive to temperature fluctuations, a property that may account for its role in regulating expression of many temperature-responsive genes (Fang & Rimsky, 2008; Stoebel *et al.*, 2008; Dillon & Dorman, 2010; Shapiro & Cowen, 2012). The formation of complex nucleoprotein structures obstructing RNA polymerase movements has generally been accounted for transcriptional arrest (Fang & Rimsky, 2008; Stoebel *et al.*, 2008; Dillon & Dorman, 2010; Shapiro & Cowen, 2012). However, positive roles of H-NS in the expression of a number of *E. coli* genes have also been reported (Johansson *et al.*, 1998; White-Ziegler *et al.*, 2000; Müller *et al.*, 2006; White-Ziegler & Davis, 2009; Park *et al.*, 2010). In this context, it has been also noted that some configurations may exist in which H-NS could stabilize a particular DNA conformation that favors the correct recognition of promoter sequences by the RNA polymerase and/or a positive transcriptional regulator, or exclude a repressor (Fang & Rimsky, 2008; Stoebel *et al.*, 2008; Singh & Grainger, 2013). *ompW* thus represents another example of a temperature-regulated gene whose expression is positively mediated by H-NS. It is noteworthy, however, that cells lacking H-NS showed reduced but not complete impairments of *ompW* transcription at 37 °C and that the additional removal of StpA was required to abolish OmpW synthesis (Fig. 2). This argues for a regulatory mechanism in which these nucleoid-binding proteins act better by stabilizing a particular DNA topology that favors the productive recognition of the *ompW* promoter by the RNA polymerase, rather than indicating an absolute requirement of these proteins for *ompW* expression.

The results above also support the notion that OmpW roles are more related to the interaction of the *E. coli* cell

with its warm-blooded hosts (McClean, 2012), and, in fact, the shielding of this colicin receptor outside the host probably increases survival advantage (Govan, 1986). Providing the various causes that can differentially modify OmpW contents in bacteria (Bina *et al.*, 2003; Hu *et al.*, 2005; Nandi *et al.*, 2005; Xu *et al.*, 2006; Gil *et al.*, 2007; Lin *et al.*, 2008, 2010; Wu *et al.*, 2009), it seems likely that both positive and negative factors concertedly act in regulating *ompW* expression. Further work is being conducted to elucidate these factors and the roles played by OmpW in bacterial physiology.

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References

Arnold T, Zeth K & Linke D (2009) Structure and function of colicin S4, a colicin with a duplicated receptor-binding domain. *J Biol Chem* **284**: 6403–6413.

Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL & Mori H (2006) Construction of *Escherichia coli* K-12 in frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* **2**: 2006–2008.

Bina J, Zhu J, Dziejman M, Faruque S, Calderwood S & Mekelanos J (2003) ToxR regulon of *Vibrio cholerae* and its expression in vibrios shed by cholera patients. *P Natl Acad Sci USA* **100**: 2801–2806.

Brambilla L, Morán-Barrio J & Viale AM (2014) The low molecular mass PBP6b (DacD) is required for an efficient GOB-18 metallo-β-lactamase biogenesis in *Salmonella enterica* and *Escherichia coli*. *Antimicrob Agents Chemother* **58**: 205–211.

Cherepanov PP & Wackernagel W (1995) Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic resistance determinant. *Gene* **158**: 9–14.

De la Cruz MA & Calva E (2010) The complexities of porin genetic regulation. *J Mol Microbiol Biotechnol* **18**: 24–36.

Dillon SC & Dorman CJ (2010) Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat Rev Microbiol* **8**: 185–195.

Ellermeier CD, Janakiraman A & Slauch JM (2002) Construction of targeted single copy *lac* fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. *Gene* **290**: 153–161.

Fang FC & Rimsky S (2008) New insights into transcriptional regulation by H-NS. *Curr Opin Microbiol* **11**: 113–120.

Gil F, Ipinza F, Fuentes J, Fumeron R, Villarreal JM, Aspée A, Mora GC, Vásquez CC & Saavedra C (2007) The *ompW* (porin) gene mediates methyl viologen (paraquat) efflux in *Salmonella enterica* serovar typhimurium. *Res Microbiol* **158**: 529–536.

Govan JR (1986) *In vivo* significance of bacteriocins and bacteriocin receptors. *Scand J Infect Dis Suppl* **49**: 31–37.

Hu WS, Li PC & Cheng CY (2005) Correlation between ceftriaxone resistance of *Salmonella enterica* serovar Typhimurium and expression of outer membrane proteins OmpW and Ail/OmpX-like protein, which are regulated by BaeR of a two-component system. *Antimicrob Agents Chemother* **49**: 3955–3958.

Johansson J, Dagberg B, Richet E & Uhlin BE (1998) H-NS and StpA proteins stimulate expression of the maltose regulon in *Escherichia coli*. *J Bacteriol* **180**: 6117–6125.

Lin J, Huang S & Zhang Q (2002) Outer membrane proteins: key players for bacterial adaptation in host niches. *Microbes Infect* **4**: 325–331.

Lin XM, Wu LN, Li H, Wang SY & Peng XX (2008) Downregulation of Tsx and OmpW and upregulation of OmpX are required for iron homeostasis in *Escherichia coli*. *J Proteome Res* **7**: 1235–1243.

Lin XM, Yang JN, Peng XX & Li H (2010) A novel negative regulation mechanism of bacterial outer membrane proteins in response to antibiotic resistance. *J Proteome Res* **9**: 5952–5959.

McClean S (2012) Eight stranded β-barrel and related outer membrane proteins: role in bacterial pathogenesis. *Protein Pept Lett* **19**: 1013–1025.

Mendoza-Vargas A, Olvera L, Olvera M *et al.* (2009) Genome-wide identification of transcription start sites, promoters and transcription factor binding sites in *E. coli*. *PLoS One* **4**: e7526.

Miller JH (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Müller CM, Dobrindt U, Nagy G, Emödy L, Uhlin BE & Hacker J (2006) Role of histone-like proteins H-NS and StpA in expression of virulence determinants of uropathogenic *Escherichia coli*. *J Bacteriol* **188**: 5428–5438.

Nandi B, Nandy RK, Sarkar A & Ghose AC (2005) Structural features, properties and regulation of the outer-membrane protein W (OmpW) of *Vibrio cholerae*. *Microbiology* **151**: 2975–2986.

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* **4**: 593–656.

Park HS, Ostberg Y, Johansson J, Wagner EG & Uhlin BE (2010) Novel role for a bacterial nucleoid protein in translation of mRNAs with suboptimal ribosome-binding sites. *Genes Dev* **24**: 1345–1350.

Pilsl H, Smajs D & Braun V (1999) Characterization of colicin S4 and its receptor, OmpW, a minor protein of the *Escherichia coli* outer membrane. *J Bacteriol* **181**: 3578–3581.

Sambrook J, Fritsch EF & Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn, Cold Spring Harbor Press, Cold Spring Harbor, NY.

Shapiro RS & Cowen LE (2012) Thermal control of microbial development and virulence: molecular mechanisms of microbial temperature sensing. *MBio* **3**: e00238–12.

Singh SS & Grainger DC (2013) H-NS can facilitate specific DNA-binding by RNA polymerase in AT-rich gene regulatory regions. *PLoS Genet* **9**: e1003589.

Sonden B & Uhlin BE (1996) Coordinated and differential expression of histone-like proteins in *Escherichia coli*: regulation and function of the H-NS analog StpA. *EMBO J* **15**: 4970–4980.

Stoebel DM, Free A & Dorman CJ (2008) Anti-silencing: overcoming H-NS-mediated repression of transcription in Gram-negative enteric bacteria. *Microbiology* **154**: 2533–2545.

White-Ziegler CA & Davis TR (2009) Genome-wide identification of H-NS-controlled, temperature-regulated genes in *Escherichia coli* K-12. *J Bacteriol* **191**: 1106–1110.

White-Ziegler CA, Villapakkam A, Ronaszeki K & Young S (2000) H-NS controls *pap* and *daa* fimbrial transcription in *Escherichia coli* in response to multiple environmental cues. *J Bacteriol* **182**: 6391–6400.

Wu L, Lin XM & Peng XX (2009) From proteome to genome for functional characterization of pH-dependent outer membrane proteins in *Escherichia coli*. *J Proteome Res* **8**: 1059–1070.

Xu C, Lin X, Ren H, Zhang Y, Wang S & Peng X (2006) Analysis of outer membrane proteome of *Escherichia coli* related to resistance to ampicillin and tetracycline. *Proteomics* **6**: 462–473.

Zhang A, Rimsky S, Reaban ME, Buc H & Belfort M (1996) *Escherichia coli* protein analogs StpA and H-NS: regulatory loops, similar and disparate effects on nucleic acid dynamics. *EMBO J* **15**: 1340–1349.

Zhou J & Rudd KE (2013) EcoGene 3.0. *Nucleic Acids Res* **41**: D613–D624.