Available online at www.sciencedirect.com
ScienceDirect
Biochimie 90 (2008) 1539–1544
BIOCHIMIE
www.elsevier.com/locate/biochi

Research paper

Porin biogenesis activates the σ^E response in *Salmonella hfq* mutants

Lionello Bossi, Danièla Maloriol, Nara Figueroa-Bossi*

Centre de Génétique Moléculaire, CNRS, avenue de la Terrasse, 91198 Gif-sur-Yvette, France

Received 26 January 2008; accepted 4 June 2008
Available online 10 June 2008

Abstract

In *Salmonella enterica*, loss of RNA chaperon Hfq promotes proteolytic cleavage of anti-σ^E factor RseA leading to the constitutive induction of the σ^E-dependent envelope stress response. Seeking to identify the source of the inducing signal, in the present study we measured RseA cleavage and σ^E-dependent transcription in strains lacking relevant outer membrane protein (*omp*) genes. We found removal of the main *Salmonella* porin, OmpD, to markedly reduce σ^E activation in *hfq* mutant cells. Subsequent removal of LamB and of OmpC further attenuated the response, indicating that different OMPs collectively contribute to the σ^E-activated phenotype. Thus, loss of Hfq-mediated regulation might cause unfolded OMPs to accumulate in the periplasm, triggering the σ^E response. These findings corroborate the role of Hfq protein as a pleiotropic regulator of OMP biogenesis in Gram-negative bacteria.

Keywords: *hfq*; σ^E; Porins; *Salmonella*

1. Introduction

In Gram-negative bacteria, conditions that perturb folding or assembly of outer membrane proteins (OMPs) activate the σ^E-dependent envelope stress response [1–3]. Under normal conditions, binding of trans-membrane protein RseA sequesters alternative sigma factor σ^E to the cytoplasmic face of the inner membrane. Accumulation of denatured or misfolded OMPs in the periplasm sets off a proteolytic cascade that destroys RseA, resulting in σ^E release. Free σ^E associates with RNA polymerase core enzyme to promote transcription of a set of loci whose products—periplasmic folding catalysts, chaperons and proteases—help bacteria recover from envelope stress [1–3]. Also part of the σ^E regulon are the genes for two small RNAs, MicA and RybB, that pleiotropically down-regulate the synthesis of several OMPs [4–8]. Since accumulation of unfolded OMPs triggers the σ^E response, MicA and RybB provide a means for an autogenous control. The event initiating RseA proteolysis is the activation of DegS protease [9]. In the resting state, DegS is kept inactive by an intramolecular obstruction involving its PDZ domain. Under conditions that perturb folding of OMPs, the exposed C-termini of these proteins bind to DegS PDZ domain, causing a conformational change that activates the enzyme [10]. Activated DegS cleaves the C-terminal periplasmic domain of RseA, which in turns stimulates further cleavage by a second membrane-associated protease and eventually results in complete RseA degradation [11].

Besides stress-induced alterations, overexpression of porin genes can activate the σ^E response. Apparently, porin overaccumulation can saturate the folding capacity of periplasmic chaperons resulting in the exposure of normally buried C-terminal sequences [9,12]. Recently, loss of RNA chaperon protein Hfq was shown to cause constitutive induction of the σ^E response [4,13]. Hfq protein intervenes in the post-transcriptional regulation of a number of *omp* genes by mediating the action of small regulatory RNAs (sRNAs) and possibly also through direct effects on *omp* mRNA translation or stability. At least two porins, LamB and OmpD, were reported to be overproduced in *hfq* mutants [4,14], raising the possibility that excess synthesis of these and/or of additional proteins is responsible for producing the DegS-activating signal in *hfq* mutant cells. This hypothesis was tested in the

* Corresponding author. Tel.: +33 1 69 82 38 11; fax: +33 1 69 82 38 77.
E-mail address: figueroa@cgm.cnrs-gif.fr (N. Figueroa-Bossi).

0300-9084/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2008.06.001

present study by analyzing the effect of deleting or disrupting relevant porin genes on RseA cleavage and on σ^E activation in the *hfq* mutant background.

2. Materials and methods

2.1. Bacterial strains

Strains used in this study (Table 1) were all derived from *Salmonella enterica* serovar Typhimurium strain MA3409, a strain LT2 derivative cured for the Gifsy-1 prophage [15]. Strains were constructed by phage P22 transduction as described [16]. Construction of deletions Δ*micA73*::*cat* and Δ*rybB77*::*cat* is described in [4] and [17], respectively. Alleles Δ*ompD78*::*aadA*, Δ*ompA84*::*aph*, Δ[*lamB–malM*]*87*::*aph*, Δ*ompF88*::*aph* and *degP1*::*aph* were constructed in the course of this work by the λ Red-mediated recombination method as implemented by Datsenko and Wanner [18]. PCR primers and plasmid DNA templates used for these constructions are listed in Table 2. When required, the antibiotic-resistance cassette introduced by recombination was excised upon transforming strains with plasmid pCP20, which expresses the Flp recombinase [19]. Allele *degP1*::*aph* was converted to a translational *lacZ* fusion following excision of the *aph* cassette and transformation with plasmid pCE40 DNA [20]. The fusion joins the *lacZ* gene to the second codon of *degP*.

Table 1
Salmonella enterica serovar Typhimurium strains used in this work

Strain[a]	Genotype[b]	Source or reference[c]
MA3409	wild-type (Gifsy-1[-])	[15]
MA7455	wild-type (Gifsy-1[-])/pKD46	[4]
MA8028	*eptB115*::MudK	[4]
MA8029	*eptB115*::MudK Δ*hfq67*::*cat*	[4]
MA8260	*rseA71*::3xFLAG-*aph*	[4]
MA8311	*rseA71*::3xFLAG-*aph* Δ*hfq67*::*cat*	[4]
MA8743	*eptB115*::MudK *ompC390*::Tn*10* Δ*hfq67*::*cat*	
MA8773	*rseA71*::3xFLAG-scar^{pSUB11} *lamB111*::MudK Δ*hfq67*::*cat*	
MA8774	*rseA71*::3xFLAG-scar^{pSUB11} *ompC390*::Tn*10* Δ*hfq67*::*cat*	
MA8779	*eptB115*::MudK Δ*micA73*::scar^{pKD3} Δ*rybB77*::*cat*	
MA8904	*eptB115*::MudK Δ*ompD78*::*aadA*	
MA8911	*rseA71*::3xFLAG-scar^{pSUB11} Δ*ompD78*::*aadA* Δ*hfq67*::*cat*	
MA8912	*rseA71*::3xFLAG-scar^{pSUB11} *lamB111*::MudK Δ*ompD78*::*aadA* Δ*hfq67*::*cat*	
MA8919	*eptB115*::MudK Δ*ompD78*::*aadA* Δ*hfq67*::*cat*	
MA8959	*rseA71*::3xFLAG-scar^{pSUB11} *ompC390*::Tn*10* Δ*ompD78*::*aadA* Δ*hfq67*::*cat*	
MA9298	*eptB115*::MudK Δ*ompA84*::scar^{pKD13} Δ*hfq67*::*cat*	
MA9354	*eptB115*::MudK Δ[*lamB-malM*]*87*::scar^{pKD4} Δ*hfq67*::*cat*	
MA9404	*eptB115*::MudK Δ[*lamB-malM*]*87*::scar^{pKD4} Δ*ompD78*::*aadA* Δ*hfq67*::*cat*	
MA9405	*eptB115*::MudK Δ[*lamB-malM*]*87*::scar^{pKD4} *ompC390*::Tn*10* Δ*hfq67*::*cat*	
MA9406	*eptB115*::MudK Δ[*lamB-malM*]*87*::scar^{pKD4} *ompC390*::Tn*10* Δ*ompD78*::*aadA* Δ*hfq67*::*cat*	
MA9499	*eptB115*::MudK Δ*ompF88*::scar^{pKD4} Δ*hfq67*::*cat*	
MA9576	*degP1*::*aph*	
MA9591	*degP1*::*lac*	
MA9603	*degP1*::*lac* Δ*hfq67*::*cat*	
MA9610	*degP1*::*lac* Δ*micA73*::scar^{pKD3} Δ*rybB77*::*cat*	

[a] All strains are derived from *Salmonella enterica* serovar Typhimurium strain MA3409. The latter is a derivative of strain LT2 cured for the Gifsy-1 prophage [15].

[b] The term "scar" denotes the DNA sequence remaining after excision of antibiotic-resistance cassette [18]. Superscript indicates the plasmid used as DNA templates in amplifying the cassette.

[c] Where not specified, the source of the strain is this work.

2.2. Media and growth conditions

Bacteria were cultured at 37 °C in liquid media or in media solidified by the addition of 1.5% (w/v) Difco agar. LB broth (1% bacto tryptone (w/v), 0.5% Difco yeast extract (w/v), 0.5% NaCl (w/v) was used as complex medium. When needed, LB medium was supplemented with 0.2% (w/v) arabinose. Antibiotics (Sigma) were included at the following final concentrations: chloramphenicol, 10 μg/ml; kanamycin monosulphate, 50 μg/ml; sodium ampicillin, 75 μg/ml; spectinomycin dihydrochloride, 80 μg/ml; tetracycline hydrochloride, 25 μg/ml. LB plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; from Sigma), 40 μg/ml, were used to monitor *lacZ* expression in bacterial colonies. Liquid cultures were grown in New Brunswick gyratory shakers and growth was monitored by measuring the optical density at 600 nm with a Milton–Roy Spectronic 301 spectrophotometer.

2.3. OMP preparation

Outer membrane proteins were prepared as described in [21] and fractionated by SDS–PAGE on a 13% acrylamide gel.

2.4. Northern blot analysis

RNA was extracted as described in [4], fractionated on 1.3% agarose–formaldehyde gels, and transferred to Hybond-N^+ membrane. RNA blots were probed with radioactively-labelled DNA oligonucleotides complementary to *ompD* mRNA (pp931: TAT ACC TCG GCT GCA TTT ACA ACG CCT GCT GCC AAC AG) and *ssrA* RNA (pp813: GCG GAG GCT AGG GAG AGA GG). RNA was analysed by Phosphorimaging using ImageQuant software.

2.5. Western blot analysis

For the detection of 3xFLAG-tagged RseA proteins, overnight LB cultures were diluted 1:100 in LB broth, or in LB broth supplemented with 0.2% (w/v) arabinose and growth resumed at 37 °C. Cells were harvested when the culture reached an OD_{600} of 0.4. Pellets from 20 ml culture aliquots were resuspended in 200 μl of Laemmli protein gel loading buffer and kept at −20 °C prior to use. Whole-cell extracts were fractioned in a 12% polyacrylamide–SDS gel and processed for immunodetection of 3xFLAG-tagged proteins as previously described [22]. Proteins separated by SDS–polyacrylamide gel electrophoresis were transferred to polyvinylidene difluoride (PVDF) membranes