Biochemical and Biophysical Research Communications 421 (2012) 296–300

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Activation of the Cpx phosphorelay signal transduction system in acidic phospholipid-deficient *pgsA* mutant cells of *Escherichia coli*

Aya Itou, Kouji Matsumoto, Hiroshi Hara *

Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University, 255 Shimo-ohkubo, Sakura-ku, Saitama-shi, Saitama-ken 338-8570, Japan

ARTICLE INFO

Article history:
Received 16 March 2012
Available online 7 April 2012

Keywords:
Phospholipid
pgsA
pssA
Rcs
Cpx
Outer membrane lipoprotein

ABSTRACT

The *pgsA* gene encodes the enzyme for the committed step in the synthesis of acidic phospholipids in *Escherichia coli*, and the *pssA* gene does the same for zwitterionic phospholipid. It has been reported that the Rcs and Cpx phosphorelay signal transduction systems are activated in *pgsA*- and *pssA*-defective mutants, respectively. In this study, we show that the Cpx system is activated also in a *pgsA* mutant, whereas the Rcs system was not activated in a *pssA* mutant. Lack of phosphatidylglycerol in *pgsA* mutants causes inadequate modification of lipoproteins, resulting in poor localization to the outer membrane. The outer membrane lipoprotein RcsF is necessary for the response of the Rcs system to various stimuli, and Rcs activation in *pgsA* mutants involves inner membrane mislocalization of this lipoprotein. The outer membrane lipoprotein NlpE, however, while necessary for the surface adhesion-induced Cpx response, was not involved in Cpx activation in the *pgsA* mutant.

1. Introduction

The membranes of *Escherichia coli* (excluding the outer leaflet of the outer membrane, which is made up of lipopolysaccharide) are composed of acidic phospholipids (phosphatidylglycerol and cardiolipin) and zwitterionic phospholipid (phosphatidylethanolamine) [1] (Fig. 1). These are synthesized from a common intermediate, CDP-diacylglycerol. The committed steps to the synthesis of acidic and zwitterionic phospholipids are catalyzed by the gene products of *pgsA* and *pssA*, respectively (Fig. 1). A *pgsA* null mutant completely lacking phosphatidylglycerol and cardiolipin has been shown to be viable if it lacks the major outer membrane lipoprotein encoded by the *lpp* gene [2–4]. A *pssA* null mutant completely lacking phosphatidylethanolamine was found to be viable if grown in the presence of divalent metal ions at high concentrations [5,6].

It has been reported that in the *pgsA* null mutant the Rcs phosphorelay signal transduction system is activated, causing a thermosensitive growth defect [7,8], and that in the *pssA* null mutant the Cpx phosphorelay signal transduction system is activated [9]. The Rcs system is composed of the sensor kinase RcsC, the phosphotransmitter YojN (also called RcsD) and the response regulator RcsB [10]. The Cpx system is composed of the sensor kinase CpxA and the response regulator CpxR [11]. These systems respond to different kinds of envelope stresses, and both are implicated in biofilm formation. We wondered if the *pgsA* mutation would cause Cpx activation and if the *pssA* mutation might cause Rcs activation. We found that the former is the case, whereas the latter is not.

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture media

The *E. coli* strains and the plasmids used for this study are listed in Table 1. For the detailed strain construction procedure, see Supplementary Table S1. Primers used for this study are listed in Supplementary Table S2. For placement of the *pssA* gene under the control of the P_{BAD} promoter, the λ InCh system [12] was used. In pAI2, a NotI restriction site was created between the termination codon and the ρ-independent transcription terminator of the *cpxP* gene. The FLK2 cassette from pTOF30 [13] was introduced into the NotI site to construct pAI3. Replacement of the corresponding chromosomal region with the cloned fragment in pAI3 resulted in operon fusion, in which *lacZ* was fused downstream of *cpxP*. Genetic and recombinant DNA procedures were based on standard methods [14,15].

Luria–Bertani (LB) medium [14], buffered LB medium [7], and M9 medium [14] were used. For plates, media were solidified with 1.5% agar. When appropriate, antibiotics were included at the following concentrations (in μg ml^{-1}) for multicopy and single-copy resistance genes, respectively: ampicillin, 50 and 20; chloramphenicol, 50 and 10; kanamycin, 50 and 20; tetracycline, 3 (for single copy); spectinomycin, 50 and 10. L-Arabinose was used at a concentration of 0.2% as the inducer of the P_{BAD} promoter. Cell

* Corresponding author. Fax: +81 48 858 3384.
E-mail address: hhara@mail.saitama-u.ac.jp (H. Hara).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.003

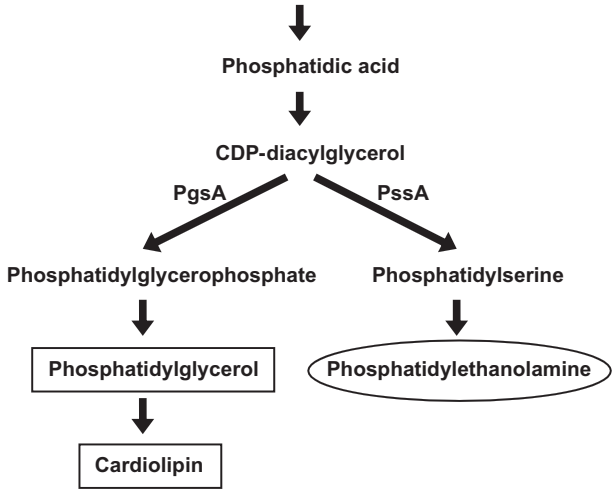

Fig. 1. The biosynthetic pathway for the major phospholipids in *E. coli*. Major acidic and zwitterionic phospholipids are enclosed in rectangles and an oval, respectively. Reactions catalyzed by *pgsA* and *pssA* gene products are indicated.

growth was monitored with a Klett-Summerson colorimeter equipped with a No. 54 filter.

2.2. Biochemical procedures

Cellular phospholipids were extracted and analyzed as described previously [16]. Densities of spots on thin layer chromatograms were quantified by High Speed TLC Scanner CS-920 (Shimadzu). The β-galactosidase assay method using *o*-nitrophenyl-β-D-galactoside as substrate and the unit definition were as described in Wang and Doi [17].

3. Results

3.1. Characterization of pgsA- and pssA-repressed cells

For this study we used strains in which *pgsA* or *pssA* is under the control of the L-arabinose-inducible P_{BAD} promoter, instead of using *pgsA* null or *pssA* null strains in which activation of the Rcs or Cpx system was first reported. The strain construction procedures were carried out in the presence of arabinose, and the activities of the signal transduction systems were tested in the absence of the inducer.

In the presence of arabinose, the *pgsA*-repressible strain UE107 and the *pssA*-repressible strain UE110 showed phospholipid composition similar to the wild type MG1655 (Table 2). In the absence of arabinose, phosphatidylglycerol and cardiolipin contents were greatly reduced in UE107, and the phosphatidylethanolamine content was significantly reduced in UE110. Although removal of the inducer did not lead to complete loss of phosphatidylglycerol in UE107 or of phosphatidylethanolamine in UE110, the growth phenotypes of these strains in the absence of arabinose were the same as those of *pgsA* null and *pssA* null strains: UE107 was thermosensitive at 42 °C, and UE110 was dependent on supplementation of a high concentration of Mg^{2+} for growth. Moreover, Rcs and Cpx signal transduction systems were activated in the *pgsA*- and *pssA*-repressed strains, respectively, (see below) just as in the corresponding null strains.

3.2. Cpx activation in the pgsA-repressed cells

The *cpxP* gene is positively regulated by the Cpx system [18]. In this study a *cpxP-lacZ* operon fusion was constructed and used for monitoring the activation of the system. Since CpxP is a regulatory component of the Cpx system [19], *cpxP* was kept intact and *lacZ*

Table 1
Bacterial strains and plasmids used for this study.

Strain or plasmid	Relevant genotype or description	Construction, source, or reference
Strains		
MG1655	Wild type	Laboratory collection
EDCM367	MG1655 Δ*lacZY*	[31]
UE107	EDCM367 *Δara714 lpp-2 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pgsA*) Δ*pgsA*::FRT[a]	This study
UE110	EDCM367 *Δara714 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pssA*) Δ*pssA10*::*cam*	This study
SG20781	MC4100 *cpsB10*::*lac*-Mu-*imm* λ	[21]
UE90	SG20781 Δ*ara714*	This study
UE46	SG20781 Δ*ara714 lpp-2 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pgsA*) *pgsA30*::*kan*	[7]
UE93	SG20781 Δ*ara714 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pssA*) Δ*pssA10*::*cam*	This study
AIT01	EDCM367 Φ(*cpxP-lacZ*)[b] Km^r Cm^s	pTOF procedure [13] using pAI3
AIT02	EDCM367 *Δara714 lpp-2 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pgsA*) Δ*pgsA*::FRT Φ (*cpxP-lacZ*) Km^r	P1(AIT01) × UE107
AIT03	EDCM367 *Δara714 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pssA*) Δ*pssA10*::*cam* Φ (*cpxP-lacZ*) Km^r	P1(AIT01) × UE110
TR51	MC4100 *cpxR1*::*spc*	[19]
AIT04	AIT01 *cpxR1*::*spc*	P1(TR51) × AIT01
AIT05	AIT02 *cpxR1*::*spc*	P1(TR51) × AIT02
AIT06	AIT03*cpxR1*::*spc*	P1(TR51) × AIT03
S330A21	W3110 *lpp-2 pgsA30*::*kan rcsA330*::IS*5 rcsC*::mini-Tn*10 cam*	[7,8]
AIT07	AIT01 *rcsC*::mini-Tn*10 cam*	P1(S330A21) × AIT01
AIT08	AIT02 *rcsC*::mini-Tn*10 cam*	P1(S330A21) × AIT02
AIT09	AIT03 *rcsC*::mini-Tn*10 cam*	P1(S330A21) × AIT03
WBS262	MC4100 *nlpE*::*spc*	[29]
AIT10	AIT01 *nlpE*::*spc*	P1(WBS262) × AIT01
AIT11	AIT02 *nlpE*::*spc*	P1(WBS262) × AIT02
AIT12	AIT03 *nlpE*::*spc*	P1(WBS262) × AIT03
Plasmids		
pTOF24	pSC101 derivative; *repA*(Ts) *sacB* Cm^r Km^r	[13]
pAI2	pTOF24 ′*cpxP fieF*′; Cm^r Km^s	This study
pTOF30	pUC18 derivative; *lacZ aph* Ap^r Km^r	[13]
pAI3	pTOF24 ′*cpxP lacZ aph fieF*′; Km^r Cm^r	This study
pHR741	pGB2 $lacI^q$ P_{204}-*djlA*	[7]

[a] FRT, FLP recombinase recognition target.
[b] Φ(*cpxP-lacZ*), operon fusion in which *lacZ* is fused downstream of *cpxP*.

Table 2
Phospholipid composition (%) of *pgsA*- and *pssA*-repressed cells.

Strain	Ara	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol	Cardiolipin	Phosphatidic acid	CDP-diacylglycerol
MG1655	–	73.2 ± 0.9	23.6 ± 0.8	3.2 ± 0.2	ND[a]	ND
UE107	+	73.0 ± 0.7	23.1 ± 0.4	4.0 ± 0.4	ND	ND
	–	86.6 ± 1.5	1.5 ± 1.4	ND	7.6 ± 4.0	4.3 ± 2.8
UE110	+	72.3 ± 2.1	24.1 ± 2.0	3.6 ± 0.3	ND	ND
	–	23.5 ± 0.6	45.7 ± 2.0	30.7 ± 1.4	ND	ND

Overnight precultures were prepared at 30 °C in LB medium in the presence or absence of 0.2% arabinose. For UE110 50 mM $MgCl_2$ was included in the medium. The precultures were diluted 250-fold into the same media, and cells were grown at 30 °C to mid-exponential phase (ca. 100 Klett units). The lipids were extracted and separated by thin-layer chromatography; spots were stained with Dittmer-Lester reagent. Spot density was quantified by High Speed TLC Scanner CS-920 (Shimadzu). The means and standard errors of three measurements are shown.

[a] ND, Not detected.

was fused downstream of the *cpxP* termination codon and upstream of the ρ-independent transcription terminator (see Section 2). In *pssA*-repressed cells the Cpx system was activated (Fig. 2A) as reported for *pssA* null cells [9]. The activation depended on CpxR. CpxR-dependent activation was also observed in the *pgsA*-repressed cells. Thus, not only zwitterionic phospholipid deficiency but also acidic phospholipid deficiency causes the Cpx activation (a preliminary report on a part of these results was presented in [20]).

3.3. The Rcs system is not activated in the pssA-repressed cells

Activation of the Rcs system was monitored with a *cpsB′-lacZ* operon fusion [21]. As reported for *pgsA* null cells [7,8] the Rcs system was activated in *pgsA*-repressed cells (Fig. 2B). By contrast, the *pssA*-repressed cells did not show Rcs activation at all. Overexpression of the *djlA* gene encoding a DnaJ-like protein is known to activate the Rcs system [22,23]. Overexpression of *djlA* from plasmid pHR741 resulted in Rcs activation in the *pssA*-repressed cells, indicating the Rcs system was intact in this strain. Thus, zwitterionic phospholipid deficiency that activates the Cpx system does not activate the Rcs system.

3.4. RcsC → CpxR cross-talk is not involved in Cpx activation in the pgsA-repressed cells

Acidic phospholipid-deficient cells showed activation of both Cpx and Rcs systems. Rcs activation has been shown to depend on the sensor kinase RcsC [7]. We suspected that RcsC → CpxR cross-talk phosphorelay might be responsible for the Cpx activation in our *pgsA* mutant. However, disruption of *rcsC* in *pgsA*-repressed cells did not abolish the Cpx activation (Fig. 2C). Thus, RcsC → CpxR cross-talk seems unlikely. We do not understand why *pgsA*-repressed cells showed higher Cpx activation in the absence of RcsC than in its presence.

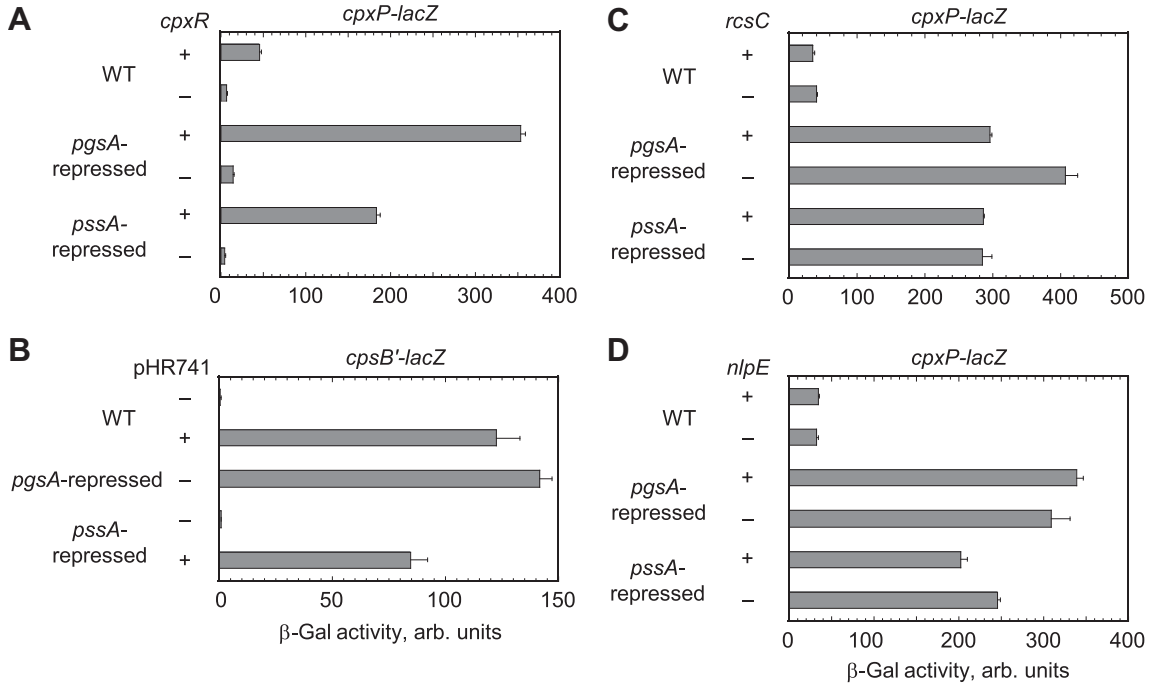

Fig. 2. Activity of Rcs and Cpx signal transduction systems monitored with *cpsB′-lacZ* and *cpxP-lacZ* operon fusions, respectively, in the *pgsA*- and *pssA*-repressed cells. Overnight precultures were prepared at 30 °C in LB medium containing 50 mM $MgCl_2$ in the absence of arabinose and diluted 100-fold into the same medium. Cells were grown to mid-exponential phase (60–90 Klett units), and the β-galactosidase activity was measured. The means and standard errors of three measurements are shown. (A) Activation of Cpx system. Strains used were AIT01 (WT *cpxR*$^+$), AIT04 (WT *cpxR*), AIT02 (*pgsA cpxR*$^+$), AIT05 (*pgsA cpxR*), AIT03 (*pssA cpxR*$^+$) and AIT06 (*pssA cpxR*). (B) Activation of Rcs system. Strains used were UE90 (WT), UE46 (*pgsA*), and UE93 (*pssA*). pHR741 carries the *djlA* gene under IPTG-inducible promoter P_{204} [7]. For pHR741-harboring strains 50 μg spectinomycin ml^{-1} and 0.8 mM isopropyl-β-D-thiogalactoside was included in the medium. (C) Possibility of cross-talk. Strains used were AIT01 (WT *rcsC*$^+$), AIT07 (WT *rcsC*), AIT02 (*pgsA rcsC*$^+$), AIT08 (*pgsA rcsC*), AIT03 (*pssA rcsC*$^+$), and AIT09 (*pssA rcsC*). (D) Possibility of the involvement of NlpE in Cpx activation. Strains used were AIT01 (WT *nlpE*$^+$), AIT10 (WT *nlpE*), AIT02 (*pgsA nlpE*$^+$), AIT11 (*pgsA nlpE*), AIT03 (*pssA nlpE*$^+$), and AIT12 (*pssA nlpE*$^+$).

3.5. The outer membrane lipoprotein NlpE is not involved in Cpx activation in the pgsA-repressed cells

Phosphatidylglycerol is the main substrate of diacylglycerol modification of lipoproteins. This modification is prerequisite for the subsequent processing and modification processes [24]. Outer-membrane lipoprotein RcsF was initially identified as an activator of the Rcs system when overproduced [25]. Later it was demonstrated that a variety of environmental and mutational stresses that activate the Rcs system are dependent on RcsF for activation [26]. Phosphatidylglycerol deficiency leads to poor modification of lipoproteins and mislocalization of outer-membrane lipoproteins to the inner membrane [4]. Mislocalization of RcsF in phosphatidylglycerol-deficient cells leads to Rcs activation, probably because of better accessibility to the sensor kinase RcsC and transmitter YojN. This is one of the causes of Rcs activation in the *pgsA*-defective mutant [27].

The outer membrane lipoprotein NlpE activates the Cpx system when overproduced [28]. Cpx activation requires NlpE at least when it is activated in response to bacterial adhesion to surfaces [29]. We thus suspected that Cpx activation in the *pgsA*-repressed cells might be due to mislocalization of NlpE to the inner membrane. However, the *pgsA*-repressed cells showed only slightly lower Cpx activation in the absence of NlpE than its presence (Fig. 2D). Thus NlpE was not responsible for the Cpx activation in the *pgsA*-repressed cells. Neither was Cpx activation in *pssA*-repressed cells dependent on NlpE, consistent with observations by Mileykovskaya and Dowhan [9].

4. Discussion

It is intriguing that the Cpx system is activated in *pgsA*-repressed cells as well as in *pssA*-repressed cells. The *pgsA*-repressed cells are deficient in acidic phospholipids, (phosphatidylglycerol and cardiolipin) and instead have a higher content of zwitterionic phospholipid (phosphatidylethanolamine) than wild-type cells. The *pssA*-repressed cells have a lower content of phosphatidylethanolamine and instead higher contents of phosphatidylglycerol and cardiolipin than wild-type cells. These shifts in membrane phospholipid composition cause changes not only in membrane surface charge but also in intrinsic curvature: phosphatidylethanolamine has a propensity to form a nonbilayer phase owing to its small polar head group, whereas phosphatidylglycerol prefers the bilayer phase. Cardiolipin also has a nonbilayer-forming propensity, but only in the presence of high concentrations of divalent cations [30]. The intrinsic curvature of the membrane with a higher content of nonbilayer-forming phosphatidylethanolamine should result in a lower packing density of head groups at the membrane surface. Although the physical properties of the membranes of the *pgsA*- and *pssA*-repressed cells are thus quite different, the alterations may directly affect the conformation of the sensor kinase CpxA or exert an indirect influence by disturbing the conformation of some periplasmic or membrane protein(s), which then activates the Cpx system. Activation of Cpx signaling in the *pgsA*-defective mutant is one of the causes of accumulation of σ^S in this mutant through partial repression of the expression of the genes for ClpXP protease [20].

Both Rcs and Cpx systems are implicated in biofilm formation [10,11]. The alterations of the properties of the membrane in the *pgsA*- and *pssA*-defective mutants may mimic the envelope perturbation brought about by environmental stresses during biofilm formation.

Acknowledgments

We are grateful to Dana Boyd and Christophe Merlin for bacterial strains and plasmids.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.003.

References

[1] I. Shibuya, Metabolic regulations and biological functions of phospholipids in *Escherichia coli*, Prog. Lipid Res. 31 (1992) 245–299.
[2] S. Kikuchi, I. Shibuya, K. Matsumoto, Viability of an *Escherichia coli* pgsA null mutant lacking detectable phosphatidylglycerol and cardiolipin, J. Bacteriol. 182 (2000) 371–376.
[3] K. Matsumoto, Dispensable nature of phosphatidylglycerol in *Escherichia coli*: dual roles of anionic phospholipids, Mol. Microbiol. 39 (2001) 1427–1433.
[4] M. Suzuki, H. Hara, K. Matsumoto, Envelope disorder of *Escherichia coli* cells lacking phosphatidylglycerol, J. Bacteriol. 184 (2002) 5418–5425.
[5] A. DeChavigny, P.N. Heacock, W. Dowhan, Sequence and inactivation of the *pss* gene of *Escherichia coli*. Phosphatidylethanolamine may not be essential for cell viability, J. Biol. Chem. 266 (1991) 5323–5332.
[6] S.K. Saha, S. Nishijima, H. Matsuzaki, I. Shibuya, K. Matsumoto, A regulatory mechanism for the balanced synthesis of membrane phospholipid species in *Escherichia coli*, Biosci. Biotechnol. Biochem. 60 (1996) 111–116.
[7] Y. Shiba, Y. Yokoyama, Y. Aono, T. Kiuchi, J. Kusaka, K. Matsumoto, H. Hara, Activation of the Rcs signal transduction system is responsible for the thermosensitive growth defect of an *Escherichia coli* mutant lacking phosphatidylglycerol and cardiolipin, J. Bacteriol. 186 (2004) 6526–6535.
[8] H. Nagahama, Y. Sakamoto, K. Matsumoto, H. Hara, RcsA-dependent and -independent growth defects caused by the activated Rcs phosphorelay system in the *Escherichia coli pgsA* null mutant, J. Gen. Appl. Microbiol. 52 (2006) 91–98.
[9] E. Mileykovskaya, W. Dowhan, The Cpx two-component signal transduction pathway is activated in *Escherichia coli* mutant strains lacking phosphatidylethanolamine, J. Bacteriol. 179 (1997) 1029–1034.
[10] Y.H. Huang, L. Ferrières, D.J. Clarke, The role of the Rcs phosphorelay in Enterobacteriaceae, Res. Microbiol. 157 (2006) 206–212.
[11] C. Dorel, P. Lejeune, A. Rodrigue, The Cpx system of *Escherichia coli*, a strategic signaling pathway for confronting adverse conditions and for settling biofilm communities?, Res Microbiol. 157 (2006) 306–314.
[12] D. Boyd, D.S. Weiss, J.C. Chen, J. Beckwith, Towards single-copy gene expression systems making gene cloning physiologically relevant: lambda InCh, a simple *Escherichia coli* plasmid-chromosome shuttle system, J. Bacteriol. 182 (2000) 842–847.
[13] C. Merlin, S. McAteer, M. Masters, Tools for characterization of *Escherichia coli* genes of unknown function, J. Bacteriol. 184 (2002) 4573–4581.
[14] J.H. Miller, A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria, Cold Spring Harbor Laboratory Press, New York, 1992.
[15] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor Laboratory Press, New York, 2001.
[16] M. Okada, H. Matsuzaki, I. Shibuya, K. Matsumoto, Cloning, sequencing, and expression in *Escherichia coli* of the *Bacillus subtilis* gene for phosphatidylserine synthase, J. Bacteriol. 176 (1994) 7456–7461.
[17] P.-Z. Wang, R.H. Doi, Overlapping promoters transcribed by *Bacillus subtilis* σ^{55} and σ^{37} RNA polymerase holoenzymes during growth and stationary phases, J. Biol. Chem. 259 (1984) 8619–8625.
[18] P.N. Danese, T.J. Silhavy, CpxP, a stress-combative member of the Cpx regulon, J. Bacteriol. 180 (1998) 831–839.
[19] T.L. Raivio, D.L. Popkin, T.J. Silhavy, The Cpx envelope stress response is controlled by amplification and feedback inhibition, J. Bacteriol. 181 (1999) 5263–5272.
[20] J. Uchiyama, Y. Sasaki, H. Nagahama, A. Itou, S. Matsuoka, K. Matsumoto, H. Hara, Accumulation of σ^S due to enhanced synthesis and decreased degradation in acidic phospholipid-deficient *Escherichia coli* cells, FEMS Microbiol. Lett. 307 (2010) 120–127.
[21] J.A. Brill, C. Quinlan-Walshe, S. Gottesman, Fine-structure mapping and identification of two regulators of capsule synthesis in *Escherichia coli* K-12, J. Bacteriol. 170 (1988) 2599–2611.
[22] W.L. Kelley, C. Georgopoulos, Positive control of the two-component RcsC/B signal transduction network by DjlA: a member of the DnaJ family of molecular chaperones in *Escherichia coli*, Mol. Microbiol. 25 (1997) 913–931.
[23] D.J. Clarke, I.B. Holland, A. Jacq, Point mutations in the transmembrane domain of DjlA, a membrane-linked DnaJ-like protein, abolish its function in promoting colanic acid production via the Rcs signal transduction pathway, Mol. Microbiol. 25 (1997) 933–944.
[24] H. Tokuda, S. Matsuyama, Sorting of lipoproteins to the outer membrane in *E. coli*, Biochim. Biophys. Acta. 1694 (2004) 5–13.
[25] F.G. Gervais, G.R. Drapeau, Identification, cloning, and characterization of *rcsF*, a new regulator gene for exopolysaccharide synthesis that suppresses the division mutation *ftsZ84* in *Escherichia coli* K-12, J. Bacteriol. 174 (1992) 8016–8022.
[26] N. Majdalani, S. Gottesman, Rcs phosphorelay: a complex signal transduction system, Annu. Rev. Microbiol. 59 (2005) 379–405.
[27] Y. Shiba, H. Miyagawa, H. Nagahama, K. Matsumoto, D. Kondo, S. Matsuoka, K.

Matsumoto, H. Hara, Exploring the relationship between lipoprotein mislocalisation and activation of the Rcs signal transduction system in *Escherichia coli*, Microbiol. 158 (2012) 1238–1248.
[28] W.B. Snyder, L.J. Davis, P.N. Danese, C.L. Cosma, T.J. Silhavy, Overproduction of NlpE, a new outer membrane lipoprotein, suppresses the toxicity of periplasmic LacZ by activation of the Cpx signal transduction pathway, J. Bacteriol. 177 (1995) 4216–4223.
[29] K. Otto, T.J. Silhavy, Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway, Proc. Natl. Acad. Sci. USA 99 (2002) 2287–2292.
[30] W. Dowhan, Molecular basis for membrane phospholipid diversity: why are there so many lipids?, Annu Rev. Biochem. 66 (1997) 199–232.
[31] C. Merlin, G. Gardiner, S. Durand, M. Masters, The *Escherichia coli metD* locus encodes an ABC transporter which includes Abc (MetN), YaeE (MetI), and YaeC (MetQ), J. Bacteriol. 184 (2002) 5513–5517.