Reassessing the role of the *Escherichia coli* CpxAR system in sensing surface contact

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Abstract

For proper biofilm formation, bacteria must have mechanisms in place to sense adhesion to surfaces. In *Escherichia coli*, the CpxAR and RcsCDB systems have been reported to sense surfaces. The CpxAR system is widely considered to be responsible for sensing attachment, specifically to hydrophobic surfaces. Here, using both single-cell and population-level analyses, we confirm RcsCDB activation upon surface contact, but find that the CpxAR system is not activated, in contrast to what had earlier been reported. Thus, the role of CpxAR in surface sensing and initiation of biofilm formation should be reconsidered.

Introduction

To ensure that the biofilm formation process is initiated only under proper conditions, bacteria must have mechanisms in place to sense adhesion to surfaces. Despite the importance of biofilms, both in disease and in technical systems, it turns out that even in the model organism *Escherichia coli* the process of surface sensing is still largely elusive [1–6]. There is evidence that in *E. coli* surface contact can be sensed with cell appendages, such as flagella and pili [7–12], but also via the two-component systems RcsCDB, rapidly activated upon contact to hydrophilic surfaces [13], and CpxAR, responding to hydrophobic [14] and possibly hydrophilic surfaces, although contradictory results have been published regarding hydrophilic surface sensing [13–15].

The Cpx system consists of an inner membrane-localized histidine kinase, CpxA, and the response regulator CpxR. Depending on the presence of inducing signals, CpxA can act either as a kinase or as a phosphatase on CpxR [16]. While the precise molecular mechanism leading to activation remains to be solved, several inducing cues were found, including extracellular copper [17], osmolarity [18], pH [19,20], envelope stress [21–26] and, as reported, surface attachment [14]. The transcription factor CpxR, in its phosphorylated form, regulates expression of a large number of genes, including biofilm-related genes [27].

With regard to induction by surface attachment, expression of CpxR-controlled genes has been reported to increase threefold within an hour after bacteria adhered to hydrophobic glass beads [14]. In addition to CpxA and CpxR, also the outer membrane lipoprotein NlpE was suggested to be required for sensing hydrophobic surface contact, and these three proteins were also needed for stable adhesion to hydrophobic surfaces [14]. A later study by Shimizu
and coworkers [15], using a similar experimental approach, reported surface sensing by CpxAR in a pathogenic *E. coli* strain. Because of the huge biofilm-related problems in both medical and technical areas [28–31], and the currently limited understanding about the initial sensing of surface contact, we aimed at further investigating the CpxAR system with single-cell analyses employing fluorescence microscopy and microfluidics.

Here, while we could confirm that RcsCDB is highly responsive to growth on a surface, we could not confirm the earlier reported response of CpxAR to surface attachment, neither with novel single-cell analyses, nor with the population-level experiments as originally done and reported [14]. Our results indicate that RcsCDB, but not CpxAR, is activated upon attachment. Thus, the role of *E. coli*’s Cpx system as a surface sensing system, as widely assumed [2,4,5,32–35], should be reconsidered.

**Results**

To investigate the single-cell response of *E. coli* to surface contact, we used microfluidics and microscopy. Specifically, bacteria were transferred from an exponential phase culture in M9 glucose medium to the microfluidic device, where they were brought in contact with the surface of the cover glass by placing a polyacrylamide gel pad on top of the cells (Fig 1A). To ensure otherwise constant conditions (apart from the surface contact), the gel pad had been equilibrated with spent medium, which was also constantly perfused over the polyacrylamide pad during the whole experiment.

To confirm that immobilization in the microfluidic setup is perceived as surface contact, *E. coli* carrying a fluorescent transcriptional reporter, controlled by the RcsAB-regulated *rcsA* promoter [13,36], were transferred to the microfluidic device, and the cells were observed by time-lapse fluorescence microscopy. The cells rapidly became highly fluorescent (Fig 1B). When the *rcsB* gene was deleted, the large increase in fluorescence was no longer observed upon surface contact (S1A Fig). Thus, the previously reported surface sensing by the Rcs system [13] was also observed in our microfluidic setup, showing that the system can be used to investigate the response of single cells to surface contact.

Towards investigating the surface response of CpxAR, we first tested the functionality of the respective reporter. Specifically, we tested induction of the two-component system by copper, a known activating signal [17]. Here, we observed a rapid increase in fluorescence in cells carrying a fluorescent transcriptional reporter under control of the CpxR-regulated *yebE* promoter [17,27,37] (S1B Fig). To exclude a global effect of copper as the cause of the induction, we also tested the reporter for the Rcs system and found that it was not induced (S1B Fig). Thus, the transcriptional reporter for CpxAR is functional.

To test whether the Cpx system responds to surface contact, similarly as the Rcs system, *E. coli* carrying the reporter plasmid were immobilized in the microfluidic device and followed in time-lapse by fluorescence microscopy. Here, we found that the fluorescence intensity remained unchanged after surface attachment (Fig 1B and 1C). When cells carried a different CpxR-controlled reporter, namely for the *cpxP* promoter [17,19,27,37], there was also no increase in fluorescence (Fig 1B and 1C). As the previous report, where the Cpx system was suggested to respond to surface contact with a threefold induction after one hour [14], had used hydrophobic surfaces, we next performed the same experiment with a cover glass that was rendered hydrophobic. Also here, even though we used the same hydrophobic dimethyldichlorosilane coating as previously [14] used, the fluorescence intensity of attached cells with the *yebE* reporter was unaffected (Fig 1B and 1C). As a control, we tested induction of this reporter by copper in the microfluidic device, where we could clearly observe increased fluorescence (S1C Fig), indicating that our experimental setup is capable of detecting activation of
CpxR. These results, where we could not find surface-induced activation of the CpxR system, neither on untreated nor on hydrophobic cover glasses, are inconsistent with the reported role of CpxAR in surface sensing.

As so far, we used glucose minimal medium and exponentially growing cells, we adjusted the growth conditions to mimic those applied by Otto and Silhavy [14]: the cells were grown until OD$_{600}$ of 2.0 in LB medium before we introduced them into the microfluidic device. We used a hydrophobic cover glass and the flow of medium over the polyacrylamide pad was spent LB to mimic the conditions in the earlier performed experiments. Also under these conditions, the fluorescence intensity of each cell at the first time point is set to 100%. Error bars show 95% confidence intervals. (c) Same as b, but excluding the reporter for the Rcs system, and with a different y-axis scaling. (d) Fluorescence intensity in surface-attached MG1655 + pPyebE-gfp (black; n = 60; 3 independent experiments), TR235 + pPyebE-gfp (red; n = 40; 2 independent experiments), MG1655 + pPcpxP-gfp (blue; n = 40; 2 independent experiments) and TR235 + pPcpxP-gfp (green; n = 53; 2 independent experiments), grown in LB medium to an OD$_{600}$ of 2.0 before introduction into the microfluidic system, with flow of spent LB medium. The fluorescence intensity of each cell at the first time point is set to 100%. Error bars show 95% confidence intervals. (e) Overview of the population-level assay. E. coli TR235 from early stationary phase LB culture were incubated with or without hydrophobic glass beads for 1 h. Unattached cells in the sample with beads were removed and discarded. Attached cells were detached by vortexing in the presence of SDS, which causes the bacteria to lyse. For both the detached sessile cells and the planktonic control, the β-galactosidase activity and total protein content were determined. (f) Comparison of β-galactosidase activity in planktonic and sessile TR235 (MC4100 λRS88[cpxR-lacZ]). Planktonic: bacteria incubated without beads for 1 h. Sessile: Bacteria that were incubated with hydrophobic beads for 1 h and that had attached. The activity was normalized to total protein content as determined from silver-stained polyacrylamide gels. The values are the mean with 95% confidence intervals (n ≥ 6).

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gene, we also saw no response in either strain to surface contact (Fig 1D). Thus, on the single-cell level we could not find any activation of the Cpx system by surface sensing.

To determine whether the negative results are caused by the different experimental setup, we repeated the original experiments that established CpxAR as a surface sensing system [14], with the same TR235 E. coli strain. Specifically, we incubated early stationary phase cells in the presence of hydrophobic glass beads for 1 h, then removed and discarded unattached cells, detached sessile bacteria by vortexing in buffer containing SDS, permeabilized them with SDS and chloroform, and carried out β-galactosidase assays, for which bacteria incubated without beads were used as the planktonic control (Fig 1E). Instead of normalization to optical density of the bacterial sample (as in the original publication), we normalized the β-galactosidase activity to total protein content, as determined from a silver-stained polyacrylamide gel. We altered the procedure, because we found that the removal of attached bacteria from the hydrophobic glass beads causes cell lysis if carried out as described. The lysis was apparent when detached cells were spun down, followed by replacement of the supernatant by fresh buffer, as this would result in an almost complete loss of β-galactosidase activity. This loss of activity indicates that the majority of the enzyme had been released from the cells. Such cell lysis also affects the quantification of the optical density. Indeed, when performing the experiments as described originally, even minute variations in sample handling, specifically during the vortexing and washing of the samples, led to highly variable results.

Instead, when exploiting the more robust normalization to protein content, experiments generated reproducible results. However, consistent with the results that we obtained from the single cell experiments, we found no difference in the expression of the reporter between planktonic and sessile cells (Fig 1F). Thus, also with the original experimental approach, slightly adapted to increase reproducibility, we were unable to observe any surface-specific response of the CpxAR system.

**Discussion**

Using both single-cell and population-level assays, we investigated surface sensing in E. coli via the CpxAR and RcsCDB systems. While we could confirm the strong induction of the Rcs system upon surface attachment, we could not identify activation of the Cpx system. The single-cell approach, involving microfluidics and fluorescence microscopy, showed a constant expression of the reporter gene following the switch from liquid culture to surface-attached growth. To exclude experimental difference as cause for this conflicting finding with an earlier report, we repeated the earlier presented population-level assay, where we also did not find any evidence for activation of the system.

One explanation for the disagreement between our results and the generally accepted view of CpxAR as a surface sensing system, could be that the original measurements on the population level had been confounded by technical factors, such as the cell lysis that we experienced upon detachment of cells from the beads. Such lysis is problematic for the measurements of both β-galactosidase activity and cell density quantification, and it may bias the results if these two measurements are not equally affected. Lysis of the samples could also lead to imprecision in the measurements if the number of intact cells would get too low. While we did not test it, we expect that the later experiments by Shimizu et al. [15] may have suffered from the same technical issues, as also here E. coli cells were detached from glass beads before determination of Cpx activity. Their normalization to colony forming units is also expected to be highly sensitive to loss of viability. Although in this later study bacteria were detached with deoxycholate, which is generally thought to be less harmful to E. coli than SDS, also here cell damage may have occurred, as deoxycholate has been shown to cause DNA damage [38], decreased growth
rate and viability [39,40], and leakage of RNA and proteins from the cell [39,41]. Overall, measurement error due to cell lysis could explain the reported activation of the Cpx system in the Otto and Silhavy paper and potentially also in the study of Shimizu et al., although the latter remains to be tested.

The previous finding that deletion of the cpxR, cpxA and nlpE genes abolished the response of the Cpx system to surface attachment [14], might instead be explained by the greatly reduced attachment of these mutants to hydrophobic surfaces. If the cells are weakly attached, as is the case in the mentioned deletion mutants [14], the lysis problems occurring at the detachment step may be alleviated, thereby removing the confounding effect. Alternatively, the mutants might have different sensitivities to stresses caused by experimental conditions, such as SDS exposure or vortexing, than the wild-type.

An alternative explanation for the different observations could be that one of the many other, non-surface-related factors induced the Cpx system in the previous studies. One possibility would be that the results had been affected by the presence of copper, which has a very strong effect on the Cpx system, even at low concentrations. In fact, Cpx induction by copper (S1B Fig) strongly resembles the dynamics found in the original surface sensing experiments [14]. Interestingly, the synthesis of the hydrophobic coating material, dimethyldichlorosilane, requires large quantities of copper [42] and possibly trace amounts might have been present in the experiments by Otto and Silhavy.

The original study, in which CpxAR had been established as a surface sensing system, is frequently cited to link the Cpx system and NlpE to adhesion and initiation of biofilm formation (e.g. [35,43–47]). As shown in this work, the connection between the Cpx system and surface sensing should be reconsidered, to avoid incorrect interpretations of experimental findings.

Materials and methods

Bacterial strains and growth conditions

The MG1655 strain carrying transcriptional fluorescent reporters for the yebE gene (pPyebE-gfp) and rcsA gene (pPrcsA-gfp) were obtained from the E. coli promoter collection [48]. The TR235 strain (MC4100 λRS88[cpxR-lacZ], [21]), which has a transcriptional reporter for the cpxR gene, was kindly provided by T.J. Silhavy. For microscopy experiments, the TR235 strain was transformed with the pPyebE-gfp plasmid. The pPcpxP-gfp reporter plasmid was constructed by amplifying the cpxP promoter region from MG1655 genomic DNA with primers TTTGGATCCCTTTAATAGGGAAGTCAGC and TTTTCTCGAGGCTTAATGAACTGACTGCCA, restriction with BamHI and XhoI and ligation into vector pUA139 [48]. The MG1655ΔrcsB strain was constructed by P1 phage transduction from the corresponding deletion strain in the Keio collection [49]. After removal of the kanamycin resistance gene, the ΔrcsB strain was transformed with the pPrcsA-gfp plasmid.

Bacteria were grown at 37°C in an orbital shaker (300 rpm), in either M9 minimal medium supplemented with 0.4% glucose or LB medium. The medium was supplemented with 25 μg/ml kanamycin for the plasmid-carrying strains. Preparation of spent medium was done by spinning down bacterial cultures at 1000 g at 4°C and subsequent filtering of the supernatant through a 0.22 μm pore-size bottle-top filter made of PES. Spent medium was always taken from cultures at the same OD600 as the culture used for the experiment.

Copper induction of Cpx and Rcs reporters

E. coli MG1655 with reporters for the Cpx (pPyebE-gfp) and Rcs (pPrcsA-gfp) systems were grown to mid exponential phase (OD 0.5–0.6) in M9 glucose medium without copper. The cultures were diluted 125–150-fold in fresh M9 glucose medium with or without 7 μM CuCl₂,
obtaining the same cell counts for all cultures, and measured at regular intervals by flow
cytometry (BD Accuri C6 flow cytometer, BD Biosciences; medium flow rate, FSC-H-threshold
8000, SSC-H threshold 500). The fluorescence intensities in the GFP channel (FL-1) were
normalized to the size of each cell, measured as the width. Each data point is the median of at
least 36,000 cells.

Copper induction of the yebE reporter was also tested in the microfluidic device. Bacteria
were exponentially grown in M9 glucose medium without copper, transferred to the polyacryl-
amide pad setup and after 75 min copper-containing (7 μM CuCl2) M9 glucose medium was
perfused.

**Preparation of hydrophobic surfaces**

Cover glasses (Menzel-Gläser #1.5) were cleaned by a procedure adapted from [50]: cover
glasses were sonicated alternatingly in absolute ethanol and 2% Hellmanex III in ultrapure
water; twice in each solvent, 30 minutes each, after which residual water was removed from
the container by 10-minute sonication in acetone, followed by rinsing of the container with
acetone twice. To apply the hydrophobic coating, the cover glasses were then incubated for 10
minutes with a 10% v/v solution of dimethyldichlorosilane in hexane, followed by extensive
rinsing with absolute ethanol, in which the cover glasses were kept for no more than two
weeks. The water contact angle (> 85˚) stayed constant during the two weeks, indicating the
stability of the coating. The silanization of 0.5 mm diameter glass beads (Sigma-Aldrich
G8772) was carried out in the same way, except for skipping the sonication steps, as the beads
had been acid-washed by the manufacturer.

**Microfluidics**

The microfluidic setup shown in Fig 1A was used. All components were prewarmed to 37˚C.
A 24 x 24 mm cover glass, either untreated (i.e. only rinsed with ethanol and ultrapure water)
or hydrophobic (see above) as described in the main text, with a thin piece of PDMS around
the edges to prevent leakage was placed in a custom-made metal holder. In the center of the
cover glass, 5 μl of bacterial culture was pipetted and immediately covered by a 1.5 mm thick
10% polyacrylamide gel pad. This pad had been extensively washed after preparation and incubu-
bated for at least one hour in spent medium. The microfluidic setup was completed by a piece
of PDMS containing a 2 x 10 mm channel that was placed on top of the pad. Using a plexiglass
frame and bolts, the setup was tightened to the metal holder. Tubings (Cole-Parmer Microbore
PTFE Tubing, EW-06417-11) were connected to the channel and spent medium was perfused
at a flow rate of 24 μl/min throughout the experiment, for which a Harvard Apparatus syringe
pump 11 Elite (#70–4505) was employed. Both the specimen and microscope were tempera-
ture-controlled to 37˚C (Life Imaging Services, The Cube and The Box).

**Fluorescence microscopy**

For image acquisition, a Nikon Eclipse Ti-E inverted microscope was used, with Nikon CFI
Plan Apo Lambda DM 100X Oil objective, CoolLED pE-2 or Lumencor Aura illumination sys-
tem (470 or 485 nm LED, respectively, for excitation of GFP) and Andor iXon 897 EM-CCD
camera. The following filters were employed: excitation filter bandpass 470/40 nm, dichroic
mirror 495 nm and emission filter 525/50 nm (AHF Analysentechnik F46-470). Focus was
maintained by Nikon’s PFS3 system. Acquisition was started within 10 min (generally ~7 min)
after the bacteria had been introduced in the microfluidic system and every 10 min phase con-
trast images and GFP signal (200 ms exposure time) were acquired at multiple positions. The
microscope was controlled by NIS Elements v4.51 software.
**Image analysis**

Image segmentation was semi-automated and handled by the ImageJ [51] plugin MicrobeJ [52], or in-house software, followed by manual inspection and correction. The detected cells were further analyzed in Matlab (R2014a, MathWorks Inc.), where the identified ROIs were applied to background-corrected GFP images. The background correction was done by first subtracting the signal intensity of an image without any bacteria, followed by division of each pixel by a correction factor to correct for uneven illumination. The correction factors were determined by smoothing the intensities on a position without cells with a 3x3 point moving average and then dividing the intensity of each pixel by the mean of all pixels.

**Population-level assay**

The assay to study CpxR activity on the population level in bead-attached cells was carried out essentially as described [14]. To plastic tubes containing 3 g of freshly prepared hydrophobic beads (prepared as described above in 'Preparation of hydrophobic surfaces'), 1 ml of OD$_{600}$ 2.0 culture of TR235 in LB was added and incubated at 37˚C. After 1 h, unattached cells were aspirated using a syringe with needle and discarded. Attached cells were then detached by addition of 1 ml Z-buffer containing 0.04% SDS, vortexing for 30 s and aspirating with a syringe with needle. Cells were lysed by addition of three drops of chloroform and vortexing for 15 s. As planktonic controls, bacteria incubated in tubes without beads were used. These control cells were spun down, resuspended in Z-buffer with 0.04% SDS and three drops of chloroform, and vortexed for 15 s. From all samples, 50 μl was set aside for determination of total protein content, and the remainder was used for the β-galactosidase assay.

**β-galactosidase assay**

The assay was carried out essentially as originally described by Miller [53]. All samples were incubated at 28˚C and the reaction was started by addition of 200 μl 4 mg/ml ONPG (Sigma-Aldrich #N1127). Reactions that had turned yellow upon visual inspection were stopped by mixing with 500 μl 1 M Na$_2$CO$_3$. The samples were spun down and the absorption at 420 nm of the supernatant was measured. The β-galactosidase activity was calculated as ($1000 \cdot A_{420}$) / (TP • t), where 'TP' is the total protein concentration in relative units and 't' is the duration of the reaction in minutes.

**Determination of total protein content**

The protein content in the β-galactosidase samples was determined from the band intensities on a silver-stained 10% SDS-PAGE gel. The gel was stained according to the procedure provided with the kit (Pierce Silver Stain Kit, #24612). The stained gel was imaged with a Fujifilm LAS-3000. In ImageJ [51], the background signal was determined and the intensities of the bands in the upper half of each gel lane were integrated, because there the bands were better resolved and with less overlap than at the bottom half. To establish the linearity of our measurements, the integrated, background-corrected band intensities were determined for a dilution range of a total protein sample of known OD$_{600}$ (S1D Fig). Using control samples that were present on multiple gels and the determined relation between OD$_{600}$ and total protein, the normalized total protein content was calculated for each sample.

**Supporting information**

S1 Fig. Control experiments. (a) Comparison of GFP expression from the Rcs-regulated rcsA promoter in wild type (black; n = 46; 2 independent experiments) and ΔrcsB cells (red; n = 40;
2 independent experiments) on untreated cover glasses, in the microfluidic device with flow of spent M9 glucose medium. The fluorescence intensity of each cell at the first time point is set to 100%. Error bars show 95% confidence intervals. (b) Effect of copper chloride on the reporters for the Cpx (pPyE_bE-gfp) and Rcs (pPrcsA_gfp) systems. Exponential phase M9 glucose cultures were diluted in fresh M9 glucose medium with or without 7 μM CuCl_2 and measured at regular intervals by flow cytometry. The fluorescence intensities were normalized to the size of each cell and shown here as the median of at least 36,000 cells. (c) Induction of the CpxR-controlled yebE reporter in the polyacrylamide pad setup by addition of copper. Cells were grown in absence of copper and after 1 h 15 min in the microfluidic device, medium containing 7 μM CuCl_2 was perfused. Note that the induction ratio is comparable to the flow cytometry experiment, but that the dynamics are different. Likely, the slower response is related to a delayed and gradually increasing exposure to copper due to diffusion through the polyacrylamide gel. (d) Linearity of the total protein content determinations. The background-corrected integrated band intensities of a number of dilutions of a total protein sample are shown as determined from a silver-stained polyacrylamide gel.

(TIF)

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References

1. O’Toole GA, Wong GCL. Sensational biofilms: Surface sensing in bacteria. Curr Opin Microbiol. Elsevier Ltd; 2016; 30: 139–146. https://doi.org/10.1016/j.mib.2016.02.004 PMID: 26968016

2. Belas R. Biofilms flagella, and mechanosensing of surfaces by bacteria. Trends Microbiol. Elsevier Ltd; 2014; 22: 517–527. https://doi.org/10.1016/j.tim.2014.05.002 PMID: 24894628

3. Geng J, Beloin C, Ghigo J-M, Henry N. Bacteria Hold Their Breath upon Surface Contact as Shown in a Strain of Escherichia coli, Using Dispersed Surfaces and Flow Cytometry Analysis. PLoS One. 2014; 9: e102049. https://doi.org/10.1371/journal.pone.0102049 PMID: 25054429

4. Tuson HH, Weibel DB. Bacteria-surface interactions. Soft Matter. 2013; 9: 4368–4380. https://doi.org/10.1039/C3SM27705D PMID: 23930134

5. Petrova OE, Sauer K. Sticky situations: key components that control bacterial surface attachment. J Bacteriol. 2012; 194: 2413–2425. https://doi.org/10.1128/JB.00003-12 PMID: 22389478

6. Dorel C. Manipulating bacterial cell fate: key role of surface-sensing and signal transduction. In: Méndez-Vilas A, editor. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Badajoz: Formatex; 2010. pp. 791–800.

7. Lele PP, Hosu BG, Berg HC. Dynamics of mechanosensing in the bacterial flagellar motor. PNAS. 2013; 110: 11839–11844. https://doi.org/10.1073/pnas.1305885110 PMID: 23818629
8. Tipping MJ, Delalez NJ, Lim R, Berry RM, Armitage JP. Load-dependent assembly of the bacterial flagellar motor. MBio. 2013; 4: e00551–13. https://doi.org/10.1128/mBio.00551-13 PMID: 23963182

9. Lee Y-Y, Belas R. Loss of Flil Alters Proteus mirabilis Surface Sensing and Temperature-Dependent Swarming. J Bacteriol. 2015; 197: 159–173. https://doi.org/10.1128/JB.02235-14 PMID: 25331431

10. Bhomkar P, Materi W, Semenchenko V, Wishart DS. Transcriptional Response of E. coli Upon FimH-mediated Fimbrial Adhesion. Gene Regul Syst Bio. 2010; 4: 1–17. PMID: 20458372

11. Zhang JP, Normark S. Induction of gene expression in Escherichia coli after pilus-mediated adherence. Science. 1996; 273: 1234–1236. PMID: 8703059

12. Otto K, Norbeck J, Larsson T, Karlsson K-A, Hermansson M. Adhesion of type 1-fimbriated Escherichia coli to abiotic surfaces leads to altered composition of outer membrane proteins. J Bacteriol. 2001; 183: 2445–2453. https://doi.org/10.1128/JB.183.8.2445-2453.2001 PMID: 11274103

13. Ferrières L, Clarke DJ. The RcsC sensor kinase is required for normal biofilm formation in Escherichia coli K-12 and controls the expression of a regulon in response to growth on a solid surface. Mol Microbiol. 2003; 50: 1665–1682. https://doi.org/10.1046/j.1365-2958.2003.03815.x PMID: 14651646

14. Otto K, Silhavy TJ. Surface sensing and adhesion of Escherichia coli controlled by the Cpx-signaling pathway. PNAS. 2002; 99: 2287–2292. https://doi.org/10.1073/pnas.042521699 PMID: 11830644

15. Shimizu T, Ichimura K, Noda M. The surface sensor NlpE of enterohemorrhagic Escherichia coli contributes to regulation of the type III secretion system and flagella by the Cpx response to adhesion. Infect Immun. 2016; 84: 537–549. https://doi.org/10.1128/IAI.00881-15 PMID: 26644384

16. Raivo TL, Silhavy TJ. Transduction of envelope stress in Escherichia coli by the Cpx two-component system. J Bacteriol. 1997; 179: 7724–7733. PMID: 9401031

17. Yamamoto K, Ishihama A. Characterization of copper-inducible promoters regulated by CpxA/CpxR in Escherichia coli. Biosci Biotechnol Biochem. 2006; 70: 1688–1695. https://doi.org/10.1271/bbb.60024 PMID: 16861934

18. Jubelin G, Vianney A, Beloin C, Ghigo J-M, Lazzaroni J-C, Lejeune P, et al. CpxR/OmpR Interplay Regulates Curli Gene Expression in Response to Osmolarity in Escherichia coli. J Bacteriol. 2005; 187: 2038–2049. https://doi.org/10.1128/JB.187.6.2038-2049.2005 PMID: 15743952

19. Danese PN, Silhavy TJ. CpxP, a stress-combative member of the Cpx regulon. J Bacteriol. 1998; 180: 831–839. PMID: 9473036

20. Clarke EJ, Voigt CA. Characterization of combinatorial patterns generated by multiple two-component sensors in E. coli that respond to many stimuli. Biotechnol Bioeng. 2011; 108: 666–675. https://doi.org/10.1002/bit.22966 PMID: 21246512

21. Raivo TL, Popkin DL, Silhavy TJ. The Cpx Envelope Stress Response Is Controlled by Amplification and Feedback Inhibition. J Bacteriol. 1999; 181: 5263–5272. PMID: 10461986

22. DiGiuseppe PA, Silhavy TJ. Signal Detection and Target Gene Induction by the CpxRA Two-Component System. J Bacteriol. 2003; 185: 2432–2440. https://doi.org/10.1128/JB.185.8.2432-2440.2003 PMID: 12670966

23. Vogt SL, Raivo TL. Just scratching the surface: an expanding view of the Cpx envelope stress response. FEMS Microbiol Lett. 2012; 326: 2–11. https://doi.org/10.1111/j.1574-6968.2011.02406.x PMID: 22092948

24. Tschauener K, Hornschmeyer P, Müller VS, Hunke S. Dynamic interaction between the CpxA sensor kinase and the periplasmic accessory protein CpxP mediates signal recognition in E. coli. PLoS One. 2014; 9: e107383. https://doi.org/10.1371/journal.pone.0107383 PMID: 25207645

25. Klein G, Lindner B, Brabetz W, Brade H, Raina S. Escherichia coli K-12 suppressor-free mutants lacking early glycosyltransferases and late acyltransferases. Minimal lipopolysaccharide structure and induction of envelope stress response. J Biol Chem. 2009; 284: 15369–15389. https://doi.org/10.1074/jbc.M900490200 PMID: 19346244

26. Klein G, Kobylak N, Lindner B, Stupak A, Raina S. Assembly of lipopolysaccharide in Escherichia coli requires the essential LapB heat shock protein. J Biol Chem. 2014; 289: 14829–14853. https://doi.org/10.1074/jbc.M113.539494 PMID: 24722986

27. Raivo TL, Leblanc SKD, Price NL. The Escherichia coli Cpx envelope stress response regulates genes of diverse function that impact antibiotic resistance and membrane integrity. J Bacteriol. 2013; 195: 2755–2787. https://doi.org/10.1128/JB.00105-13 PMID: 23564175

28. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284: 1318–1322. https://doi.org/10.1126/science.284.5418.1318 PMID: 10394980

29. Potera C. Forging a Link Between Biofilms and Disease. Science. 1999; 283: 1837–1839. PMID: 10206887
The Escherichia coli CpxAR system does not sense surface contact.

30. Jacobsen SM, Stickler DJ, Mobiley HLT, Shirtliff ME. Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008; 21: 26–59. https://doi.org/10.1128/CMR.00019-07 PMID: 18202436

31. Subramani A, Hoek EMV. Biofilm formation, cleaning, re-formation on polyamide composite membranes. Desalination. Elsevier B.V.; 2010; 257: 73–79. https://doi.org/10.1016/j.desal.2010.03.003

32. Raivio TL. Everything old is new again: An update on current research on the Cpx envelope stress response. Biochim Biophys Acta. 2014; 1843: 1529–1541. https://doi.org/10.1016/j.bbamcr.2013.10.018 PMID: 24192421

33. Persat A, Nadell CD, Kim MK, Ingremel A, Siryporn A, Drescher K, et al. The mechanical world of bacteria. Cell. Elsevier Inc.; 2015; 161: 988–997. https://doi.org/10.1016/j.cell.2015.05.005 PMID: 26000479

34. Breland EJ, Eberly AR, Hadjifrangiskou M. An Overview of Two-Component Signal Transduction Systems Implicated in Extra-Intestinal Pathogenic E. coli Infections. Front Cell Infect Microbiol. 2017; 7: 162. https://doi.org/10.3389/fcimb.2017.00162 PMID: 28536675

35. Laloux G, Collet JF. Major Tom to ground control: How lipoproteins communicate extracytoplasmic stress to the decision center of the cell. J Bacteriol. 2017; 199: e00216–17. https://doi.org/10.1128/JB.00216-17 PMID: 28674071

36. Wehland M, Bernhard F. The RcsAB box. Characterization of a new operator essential for the regulation of exopolysaccharide biosynthesis in enteric bacteria. J Biol Chem. 2000; 275: 7013–7020. https://doi.org/10.1074/jbc.275.10.7013 PMID: 10702265

37. Price NL, Raivio TL. Characterization of the Cpx regulon in Escherichia coli strain MC4100. J Bacteriol. 1987; 53: 1944–1946. PMID: 3310888

38. Merritt ME, Donaldson JR. Effect of bile salts on the DNA and membrane integrity of enteric bacteria. J Med Microbiol. 2009; 58: 1533–1541. https://doi.org/10.1099/jmm.0.014092-0 PMID: 19762477

39. D’Mello A, Yotis WW. The Action of Sodium Deoxycholate on Escherichia coli. Appl Environ Microbiol. 1987; 53: 1944–1946. PMID: 3310888

40. de Jesus MC, Urban AA, Marasigan ME, Barnett Foster DE. Acid and bile-salt stress of enteropathogenic Escherichia coli enhances adhesion to epithelial cells and alters glycolipid receptor binding specificity. J Infect Dis. 2005; 192: 1430–1440. https://doi.org/10.1086/462422 PMID: 19103922

41. Danilevich VN, Petrovskaia LE, Grishin E V. A highly efficient procedure for the extraction of soluble proteins from bacterial cells with mild chaotrophic solutions. Chem Eng Technol. 2008; 31: 904–910. https://doi.org/10.1002/ceat.200800024

42. Ward WJ, Ritzer A, Carroll KM, Flock JW. Catalysis of the Rochow Direct Process. J Catal. 1986; 100: 240–249. https://doi.org/10.1016/0021-9517(86)90089-8

43. Dorel C, Lejeune P, Rodrigue A. The Cpx system of Escherichia coli, a strategic signaling pathway for confronting adverse conditions and for settling biofilm communities? Res Microbiol. 2006; 157: 306–314. https://doi.org/10.1016/j.resmic.2005.12.003 PMID: 16487683

44. Hirano Y, Hossain MM, Takeda K, Tokuda H, Miki K. Structural Studies of the Cpx Pathway Activator NlpE on the Outer Membrane of Escherichia coli. Structure. 2007; 15: 963–976. https://doi.org/10.1016/j.str.2007.06.014 PMID: 17698001

45. Lee H-W, Koh YM, Kim J, Lee J-C, Lee Y-C, Seol S-Y, et al. Capacity of multidrug-resistant clinical isolates of Acinetobacter baumannii to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect. European Society of Clinical Infectious Diseases; 2008; 14: 49–54. https://doi.org/10.1111/j.1469-0691.2007.01842.x PMID: 18005176

46. Lacanna E, Bigosch C, Kaever V, Boehm A, Becker A. Evidence for Escherichia coli Diguanylate Cyclase DgcZ Interlinking Surface Sensing and Adhesion via Multiple Regulatory Routes. O’Toole GA, editor. J Bacteriol. 2016; 198: 2524–2535. https://doi.org/10.1128/JB.00320-16 PMID: 27402625

47. Persat A. Bacterial mechanotransduction. Curr Opin Microbiol. Elsevier Ltd; 2017; 36: 1–6. https://doi.org/10.1016/j.mib.2016.12.002 PMID: 28068612

48. Zaslaver A, Bren A, Ronen M, Itzkovitz S, Kikoin I, Shavit S, et al. A comprehensive library of fluorescent transcriptional reporters for Escherichia coli. Nat Methods. 2006; 3: 623–628. https://doi.org/10.1038/nmeth895 PMID: 16862137

49. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, et al. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol. 2006; 2: 240–249. https://doi.org/10.1038/msb4100050

50. Tanner NA, van Oijen AM. Visualizing DNA Replication at the Single-Molecule Level. Walter NG, editor. Methods in Enzymology. Elsevier Inc.; 2010. https://doi.org/10.1016/S0076-6879(10)75011-4

51. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. Nature Publishing Group; 2012; 9: 671–675. https://doi.org/10.1038/nmeth.2089 PMID: 22930834
52. Ducret A, Quadokus EM, Brun Y V. MicrobeJ, a tool for high throughput bacterial cell detection and quantitative analysis. Nat Microbiol. Nature Publishing Group; 2016; 1: 16077. https://doi.org/10.1038/nmicrobiol.2016.77 PMID: 27572972

53. Miller JH. Experiments in Molecular Genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1972.