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1 **Stress responses, outer membrane permeability control and antimicrobial resistance in**  
2 **Enterobacteriaceae**

3

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14

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17

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19

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21

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23 lipopolysaccharide (LPS), antimicrobial resistance (AMR), multidrug resistance (MDR),  
24 envelope stress response (ESR), two-component system (TCS), small regulatory RNA  
25 (sRNA).

26

27 **Abstract**

28 Bacteria have evolved several strategies to survive a myriad of harmful conditions in the  
29 environment and in hosts. In Gram-negative bacteria, responses to nutrient limitation,  
30 oxidative or nitrosative stress, envelope stress, exposure to antimicrobials and other growth-  
31 limiting stresses have been linked to the development of antimicrobial resistance. This results  
32 from the activation of protective changes to cell physiology (decreased outer membrane  
33 permeability), resistance transporters (drug efflux pumps), resistant lifestyles (biofilms,  
34 persistence) and/or resistance mutations (target mutations, production of antibiotic  
35 modification/degradation enzymes). In targeting and interfering with essential physiological  
36 mechanisms, antimicrobials themselves are considered as stresses to which protective  
37 responses have also evolved. In this review, we focus on envelope stress responses that affect  
38 the expression of outer membrane porins and their impact on antimicrobial resistance. We  
39 also discuss evidences that indicate the role of antimicrobials as signaling molecules in  
40 activating envelope stress responses.

## 41 **Introduction**

42 Antimicrobial resistance (AMR) is broadly recognized as a growing threat for human health  
43 [1, 2, 3]. As such, increasing antibiotic treatment failures due to multidrug resistant (MDR)  
44 bacteria have stirred the urgent need to better understand the underlying molecular  
45 mechanisms and promote innovation with the development of new antibiotics and alternative  
46 therapies [4, 5]. The efficacy of antibacterial compounds depends on their capacity to reach  
47 inhibitory concentrations at the vicinity of their target. This is particularly challenging for  
48 drugs directed against Gram-negative bacteria, which exhibit a complex envelope comprising  
49 two membranes and transmembrane efflux pumps [6, 7]. The Gram-negative envelope  
50 comprises an inner membrane (IM), which is a symmetric phospholipid bilayer; a thin  
51 peptidoglycan (PG) layer ensuring the cell shape; and an outer membrane (OM) that is an  
52 asymmetric bilayer, composed of an inner leaflet of phospholipids and an outer leaflet of  
53 lipopolysaccharide (LPS) [8]. The OM is a barrier to both hydrophobic and hydrophilic  
54 compounds, including necessary nutrients, metabolic substrates and antimicrobials, but  
55 access is provided by the water filled  $\beta$ -barrel channels called porins [6, 9, 10, 11, 12]. In  
56 *Escherichia coli*, the channels of the general porins OmpF and OmpC are size restricted and  
57 show a preference for passage of hydrophilic charged compounds, including antibiotics such  
58 as  $\beta$ -lactams and fluoroquinolones. These porins are conserved throughout the phylum of  $\gamma$ -  
59 proteobacteria [13]. Additionally, tripartite RND (Resistance-Nodulation-cell Division)  
60 efflux pumps, such as AcrAB-TolC in *E. coli*, play a major role in removing antibiotics from  
61 the periplasm [7, 12]. Not surprisingly, MDR clinical isolates of *Enterobacteriaceae*  
62 generally exhibit porin loss and/or increased efflux, which act in synergy to reduce the  
63 intracellular accumulation of antibiotics below the threshold that would be efficient for  
64 activity [10].

65 Given the importance of the OM in controlling the uptake of beneficial as well as toxic  
66 compounds, one can expect that the expression of porins depends on environmental stresses  
67 and is well-coordinated at the transcriptional and post-transcriptional levels [10, 14-17]. In  
68 this review, we will address the porin-mediated influx of antibiotics and give a perspective on  
69 the factors, including major regulatory pathways and antibiotic stresses, which control porin  
70 expression in *E. coli* and closely relative *Enterobacteriaceae*. Additionally, we will discuss  
71 the recent clinical data that illustrate the bacterial strategies using porins modifications to  
72 limit antibiotic entry.

73

## 74 **Antibiotic stresses**

75 Bacteria are present in a wide range of environments in which they are exposed to diverse  
76 toxic compounds or growth-limiting conditions. These include antibiotics used in the medical  
77 environment and agricultural settings. The last few decades have been marked by the constant  
78 increase of (multi)drug resistant clinical isolates to which we responded by increasing  
79 antibiotic dosing. Therefore, antibiotics are present almost everywhere at different  
80 concentrations [18]. Although MDR still emerges from bacterial exposure to antibiotic  
81 concentrations that are higher than the minimal inhibitory concentrations (MIC, defined as  
82 the lowest concentration of a drug that inhibit bacterial growth in defined laboratory  
83 conditions), the effects of subinhibitory concentrations on bacterial physiology and AMR was  
84 mostly disregarded. Importantly, studies in this field have shown that low antibiotic  
85 concentrations affect bacteria at least at four different levels: as selectors of resistance (by  
86 enriching resistant bacteria within populations and selecting for *de novo* resistance mutations)  
87 [19]; (ii) as contributors of genetic and phenotypic heterogeneity [20]; (iii) as intercellular  
88 signals [21]; (iv) as inducers of persistence [22]. In this regard, Viveiros and colleagues have  
89 demonstrated the induction of high-level resistance to tetracycline (TET) in susceptible *E.*  
90 *coli* K12 obtained by gradual, step-wise increase exposure to subinhibitory concentrations of  
91 the antibiotic [23]. Increased expression of the AcrAB efflux pump was found responsible for  
92 resistance to TET, which could also be reversed by the use of the efflux pump inhibitor  
93 phenylalanine-arginine- $\beta$ -naphthylamide (PA $\beta$ N). Interestingly, the TET-resistant strain also  
94 exhibited MDR due to repression of OmpF and OmpC expression [24]. Important questions  
95 arise from this and other related studies. First is whether the target for signaling resistance is  
96 the same as the target that is inhibited by the antibiotic. In case the antibiotic itself but not a  
97 secondary metabolite is the signaling molecule, this could be determined by examining  
98 whether the response is alleviated by a target mutation that prevents drug binding. Second is  
99 whether and how the antibiotic (or a secondary metabolite) interferes with the ESRs  
100 described above. Here, comparative transcriptomics between susceptible and resistant strains  
101 would be a valuable tool to answer this question.

102

## 103 **Global regulators**

104 In *Enterobacteriaceae*, the development of MDR is under positive regulation by global  
105 transcriptional activators that include members of Ara/XylS superfamily such as MarA,  
106 RamA (absent in *E. coli*) and Rob as well as the oxidative stress regulon SoxSR [10, 25-29].  
107 Mutations in the corresponding genes are well-documented and induce the overproduction of

108 efflux pumps with concomitant repression of porin expression both directly and indirectly  
109 [10]. These mechanisms are reviewed in details in Davin *et al.* [10]. Negative regulation by  
110 repressors of porins also plays a major role. OmpX is a small OM channel [30], of which  
111 overexpression is associated with a decreased expression of Omp36 (the OmpC ortholog of  
112 *Enterobacter aerogenes*) and a decreased susceptibility to  $\beta$ -lactams [31, 32]. Studies have  
113 indicated that expression OmpX itself is controlled by a number of environmental factors  
114 including salicylate via MarA and paraquat via SoxS [33] A very rapid MarA-dependent  
115 response pathway for upregulation of *ompX* has been shown to occur within 60-120 min upon  
116 cell exposure to salicylate [32]. This work by Dupont *et al.* identified dramatic decrease in  
117 OmpF levels, as a first line of defense, with simultaneous development of resistance to  $\beta$ -  
118 lactams and fluoroquinolones by altering OM permeability.

119

### 120 **Envelope stress responses**

121 All living organisms have stress responses that allow them to sense and respond to  
122 environmental damaging conditions by remodeling gene expression. As such, Gram-negative  
123 bacteria possess stress responses that are uniquely targeted to the cell envelope, including  
124 membranes and cell wall. These envelope stress responses (ESRs) are the EnvZ/OmpR,  
125 CpxAR (Cpx), BaeRS, and Rcs phosphorelays, the stress responsive alternative sigma factor  
126  $\sigma^E$ , and the phage shock response [34-37] in *E. coli* and closely related *Enterobacteriaceae*.  
127 Each of these ESRs is activated following the perturbation of particular components of the  
128 envelope or exposure to particular environmental stresses. Although ESRs are important for  
129 reacting to damaging conditions, stress proteins also play important roles in the maintenance  
130 of basic cellular physiology [38, 39]. This is particularly true for the  $\sigma^E$ -dependent stress  
131 response in *E. coli*, as the *rpoE* gene, which encodes  $\sigma^E$ , is essential for viability [40]. Here,  
132 we will essentially focus on ESRs that impact on AMR by regulating porin expression  
133 together with many other targets (regulons) — namely EnvZ/OmpR, Cpx and  $\sigma^E$  (see below  
134 and key figure). Additionally, with the recent highlights and advances in RNA-based  
135 techniques [41], the repertoire of small regulatory RNAs (sRNAs) has vastly increased so as  
136 to and their impact on the OM is continuously emerging [15, 17]. sRNAs alter gene  
137 expression, allowing fast adjustment to different growth conditions [42]. Noteworthy, ESRs  
138 are often interconnected, regulate and are regulated by sRNAs in order control target genes  
139 both at the transcriptional and post-transcriptional levels [15-17, 43, 44] (see below and key  
140 figure).

141 Osmolarity was one of the earliest stresses described to influence OmpF and OmpC  
142 expression via the EnvZ/OmpR two-component system (TCS) [45, 46]. EnvZ is a membrane-  
143 bound sensor kinase, and OmpR is a cytosolic response regulator, which binds to the  
144 promoter region of the porin genes. Upon activation, EnvZ autophosphorylates and the high  
145 energy phosphoryl group from EnvZ is subsequently transferred to a conserved Asp residue  
146 on OmpR. Phosphorylated OmpR (OmpR~P) serves as a transcription factor that  
147 differentially modulates the expression of the *ompF* and *ompC* porin genes [45]. At low  
148 osmolarity, high levels of OmpR~P activates *ompF* transcription, whereas at high osmolarity,  
149 low levels of OmpR~P represses *ompF* transcription and activates *ompC* transcription [47].  
150 This differential production of OmpF and OmpC is consistent with that in high osmolarity  
151 environments, such as in the hosts where nutrients are abundant the least permeable pore  
152 channel OmpC is predominant, thus limiting the uptake of toxic bile salts; whereas in low  
153 osmolarity environments where nutrients are scarce, the most permeable pore channel OmpF  
154 is expressed [6]. OmpF and OmpC transcriptional regulation by EnvZ/OmpR is also triggered  
155 by local anesthetics, pH, and nutrient limitation [46].

156 Accumulation of misfolded OM proteins in the periplasm, presumably reflecting problems in  
157 protein assembly or transport across the IM, can be detected by regulatory sensors that  
158 activate either the Cpx TCS or the alternative sigma factor  $\sigma^E$ .  $\sigma^E$  and Cpx are the two major  
159 regulation pathways that control the envelope integrity with overlapping regulon members  
160 [48-51] but respond to different inducing cues [35]. It is possible that these poorly defined  
161 signals (see below) act by causing accumulation of misfolded proteins. However, misfolded  
162 proteins are not the inducing signal *per se*, as some induce  $\sigma^E$  but not Cpx and *vice versa*.  
163 Recent studies rather suggest that Cpx responds to IM perturbations, while  $\sigma^E$  is activated by  
164 signals at the OM. The Cpx system comprises the CpxA sensor kinase and response regulator  
165 CpxR. Envelope stresses including alkaline pH, periplasmic protein misfolding, IM  
166 abnormalities such as misfolded transporters or accumulation of the lipid II precursor, induce  
167 the dissociation of the accessory protein CpxP from CpxA, trigger CpxA-mediated  
168 phosphorylation of CpxR, and altered expression of protein foldases and proteases,  
169 respiratory complexes, IM transporters, and cell wall biogenesis enzymes [37, 48, 49], all of  
170 which affect resistance to a number of antibiotics, particularly aminoglycosides and  $\beta$ -  
171 lactams [37, 49, 52-54]. The Cpx-mediated regulation of porins occurs at several levels. At  
172 the transcriptional level, CpxR~P has been shown to bind directly the *ompF* and *ompC*  
173 promoters [55]. More recently, it has been found that the small IM protein MzrA connects

174 Cpx and EnvZ/OmpR [56]. In this pathway and upon the activation of Cpx, MzrA interacts  
175 directly with EnvZ, which in turn, stabilizes OmpR~P [57]. In sensing different signals, the  
176 interconnection between Cpx and EnvZ/OmpR allows cells to adapt to diverse environmental  
177 stresses. Finally, although Cpx contributes to AMR by regulating a number of genes [37, 49,  
178 52-54], its precise role and that of other TCSs in the development of MDR in clinical isolates  
179 are still poorly documented [58]. On the other hand, the stress responsive sigma factor  $\sigma^E$  is  
180 induced by stresses that disturb the OM and its regulon members comprise genes that  
181 facilitate the biogenesis of OM components, including proteins, lipoproteins and LPS [59-  
182 67]. In the absence of inducing signals,  $\sigma^E$  is held at the cytoplasmic side of the IM by the  
183 anti-sigma factor RseA. At the periplasmic side of the IM, RseB binds to RseA, thus  
184 enhancing the inhibition of  $\sigma^E$ . Upon activation,  $\sigma^E$  is released from RseA by a proteolytic  
185 cascade that starts with the sequential degradation of the periplasmic and transmembrane  
186 domains of RseA by DegS and RseP, respectively, followed by the degradation of the  
187 cytoplasmic domain of RseA by ClpXP [68]. Interestingly, proteolysis of RseA is triggered  
188 by the binding of a conserved peptide found at the C-terminus of OM proteins, which is  
189 normally buried in folded porin trimers, to DegS in conjunction with the release of RseB  
190 from RseA upon binding of LPS intermediates [69, 70]. Of note, the  $\sigma^E$ -dependent repression  
191 of porin synthesis only occurs at the post-transcriptional level, wherein base-pairing sRNAs  
192 inhibits translation of *omp* mRNAs (see below) in order to maintain the envelope homeostasis  
193 under stress conditions, as porins are major abundant proteins under normal growth  
194 conditions [6].

195 The post-transcriptional repression of OmpF by the sRNA MicF has been discovered in 1984  
196 [71-73]. This 93-nucleotide (nt) RNA is transcribed in the opposite direction to the *ompC*  
197 gene and acts by direct base-pairing to a region that encompasses the ribosome binding site  
198 (RBS) and the start codon of the *ompF* mRNA, thus preventing translation initiation [74].  
199 The expression of the MicF sRNA itself is subjected to multiple signals and regulatory  
200 pathways [75]. Positive regulation includes EnvZ/OmpR in high osmolarity conditions [76],  
201 SoxS in response to oxidative stress [77] and MarA in response to antibiotic stress [25]. The  
202 109-nt MicC sRNA has been identified more recently and shown to repress OmpC by direct  
203 base-pairing to a 5' untranslated region of the *ompC* mRNA [78]. Interestingly, *micC* is  
204 transcribed in the opposite direction to the *ompN* gene that encodes a quiescent porin  
205 homologous to OmpF and OmpC [79]. We have recently shown that *ompN* and *micC* are  
206 subjected to dual regulation upon exposure to certain antimicrobials such as  $\beta$ -lactams in a



207  $\sigma^E$ -dependent manner [80]. This is consistent with that *ompN-micC* and *ompC-micF* share  
208 similar genetic organization and that *ompC* and *micF* are co-induced under specific  
209 conditions (i. e. high osmolarity via EnvZ/OmpR). The last decade has been marked by the  
210 identification and characterization of several sRNAs. These are differentially expressed and  
211 have been assigned to various important regulatory pathways in *E. coli* and *Salmonella*.  
212 Interestingly, many pathways regulate and are regulated by sRNAs [43, 44]. A prime  
213 example is EnvZ/OmpR, which activates the expression of MicF (that target *ompF*), OmrA  
214 and OmrB (that target *ompT* and mRNA of OM channels for iron-siderophore complexes)  
215 [81]; OmrA and OmrB, in turn, repress the translation of the *ompR* mRNA, creating a  
216 negative feedback loop [82]. Others examples include the well-conserved  $\sigma^E$ -regulated  
217 sRNAs RybB (that target *ompC* and *lamB* in *E. coli*; *ompN* and *ompW* in *Salmonella*), MicA  
218 (*ompA*), RseX (*ompC* and *ompA*), CyaR (*ompX*) and MicL (that represses translation of the  
219 major OM lipoprotein Lpp) [43, 66, 83-90] (key figure). Of note all these sRNAs are *trans*-  
220 acting, function by imperfect base pairing with multiple mRNA targets and require the help  
221 of the RNA chaperone Hfq [15-17].

222

### 223 **Porin alterations in clinical isolates**

224 Combined regulations contributed by different stressors leads to hampering of the drug  
225 accumulation inside cells under the threshold for bacterial death. In one such study in *K.*  
226 *pneumoniae*, preferential expression of OmpK37 was detected in porin-deficient strains [92].  
227 Amino acid sequencing showed that OmpK37 is highly homologous to quiescent porins  
228 OmpS2 from *Salmonella enterica* serovar Typhimurium and OmpN from *E. coli*. Liposome  
229 swelling assay with purified porins determined that OmpK37 also has a narrower pore, which  
230 was responsible for higher MICs of cefotaxime and ceftazidime antibiotics because of lower  
231 drug diffusion. A very recent study identified mutation in the *pho* regulon of an extensively  
232 drug resistant strain of *K. pneumoniae* demonstrating downregulation of *phoE* gene by  
233 mutations in *phoR* and *phoB*. Here the PhoE porin, which is normally involved in phosphate  
234 transport, promotes restoration of ceftazidime and carbapenem resistance [93]. This is an  
235 interesting example of a regulatory mutation that effects porin expression, and clinically  
236 favors AMR under antibiotic therapy.

237 A wide array of chemicals including disinfectants and antibiotics has been shown to modulate  
238 the OM permeability including expression of porins [94]. In addition, several studies have

239 described the effect of imipenem on porin loss or loss of function mutations in clinical  
240 isolates of *Enterobacteriaceae* [58, 95-100].

241 Porins are trimers of 16-stranded  $\beta$ -barrels, each monomer formed of a central channel  
242 constricted by loop 3 that folds inward, thereby restricting the size of the channel. The  
243 presence of acidic residues in loop 3 facing a cluster of basic residues on the opposite side of  
244 the pore creates a strong transversal electric field [6, 101, 102]. This so-called eyelet or  
245 constriction region determines the channel size and ion selectivity, with OmpF being more  
246 permeable than OmpC. This latter observation was first attributed to the OmpC pore being  
247 slightly more constricted in this porin compared to OmpF [101, 102]. Although the two  
248 porins share high sequence similarity, the pore interior is more negative in OmpC than in  
249 OmpF [102]. This can also account for the low permeability of OmpC for anionic  $\beta$ -lactams  
250 [103, 104]. Moreover, the replacement of all ten titratable residues that differ between OmpC  
251 and OmpF in the pore-lining region leads to the exchange of antibiotic permeation properties  
252 [105]. Together, these structural and functional data clearly demonstrate that the charge  
253 distribution at pore linings, but not pore size, is a critical parameter that physiologically  
254 distinguishes OmpC from OmpF.

255 Functional mutations in porin genes leading to reduced permeability are another strategy  
256 found in MDR bacteria. In two documented cases,  $\beta$ -lactam-resistant clinical isolates of *E.*  
257 *aerogenes* contained Omp36 (an OmpC homologue) that carried the mutation G112D in L3  
258 [106, 97]. The homologous mutation G119D in OmpF of *E. coli* narrows the size of the  
259 channel as the large side chain of Asp protrudes into the channel lumen and confers a drastic  
260 reduction in  $\beta$ -lactam susceptibility [107]. Consistently, the Omp36 G112D mutant of *E.*  
261 *aerogenes* was characterized by a 3-fold decrease in ion conductance and a significant  
262 decrease in cephalosporin sensitivity (*e. g.* MICs of cefotaxime, ceftazidime, cefepime and  
263 ceftazidime were 7 to 9 fold higher in the clinical isolate as compared to that in a sensitive  
264 reference strain) and a cross resistance to carbapenems [106, 97]. Recent studies also found a  
265 series of OmpC mutants that were isolated from a patient with chronic *E. coli* infection and  
266 additive mutations that conferred increased resistance to a variety of antibiotics, including  
267 cefotaxime, ceftazidime, imipenem, meropenem and ciprofloxacin [108, 109]. Low *et al.*  
268 demonstrated that subtle changes in OmpC in clinical isolates of *E. coli* altered antibiotic  
269 permeability and thus cell viability [108]. Seven isolates collected over a two year clinical  
270 treatment exhibited increased levels of antibiotic resistance. These isolates exhibited the same  
271 two mutations (D18E and S274F) in the OmpC porin with increased levels of antibiotic

272 resistance, thus pointing towards the possible functional role of these mutations in antibiotic  
273 influx.

274 It is worthwhile to note that from our knowledge, porin mutations causing reduced  
275 permeability have only been described in OmpC-type porins in *E coli* and *E aerogenes*.  
276 Interestingly, this type of porin is expressed under high osmolarity, the same environment the  
277 bacteria encounters the hosts. This gives an essential outlook on the host induced  
278 modifications that possibly occurs in these pathogens during infection. Heeding to this sort of  
279 information can be highly beneficial for designing drugs with an improved diffusion across  
280 the bacterial outer membrane.

281

## 282 **Conclusion**

283 It is noteworthy that the sRNA-mediated stress response mechanism has multiple benefits for  
284 bacteria as compared to regulation by protein. Since sRNAs are produced during  
285 transcription, the later stages of translation and post translational modification processes in  
286 the cell is completely skipped proving to be time and energy efficient for the cell. Not to  
287 forget the energy saved in porin assembly and discarding of misfolded proteins, which in  
288 itself can induce another stress response mechanism.

289 Decreased porin expression has been observed as a rapid response to toxic molecules and  
290 antibiotics within less than 60 minutes. Many sRNAs act at the post-transcriptional level,  
291 which ensures a rapid response to stressful conditions. In addition, the versatility of sRNAs  
292 ensures another level of gene regulation along with protein transcriptional regulators, thus  
293 contributing to an additional layer of tighter regulation. Taking into account the major role of  
294 the CpxAR and EnvZ/OmpR regulators in response to stressors such as antibiotics, it will be  
295 interesting to develop some assays allowing the detection of these kinds of mutations inside  
296 clinical isolate. This original diagnostic maybe used for determining the prevalence of these  
297 regulation events in clinical strain that have undergone antibiotic stress.

298 Targeting the early transcriptional step of antibiotic stress response regulatory mechanism is  
299 much more logical, especially when we have reports of OMP expression being regulated  
300 (both up and downregulation) within 60 minutes of stress appearance [32]. This will  
301 especially promote bypassing of aforementioned mutations in porins in clinical strains that  
302 are selected during antibiotic treatment. Targeting of sRNA or sRNA regulators such as  
303 MicF or Hfq can rejuvenate failing antimicrobial therapies in regards with membrane  
304 impermeability. They can be original targets for increasing the efficiency of existing drugs by  
305 providing fitness reduction in bacteria. As of now, a cyclic peptide RI20 has been identified

306 to inhibit Hfq-mediated repression of gene, by binding with proximal binding site of Hfq  
307 [110]. Another approach will be to inhibit sRNA interfering with porin expression that is  
308 involved in drug translocation. Recently, a small molecule was used to target human  
309 microRNA (miR)-525 precursors as an anti-cancer strategy [111]. This promising discovery  
310 can be repeated in bacteria for manipulating sRNA levels, which may save the failing  
311 antibiotic therapies.

312 Predictability of an efficient drug based on the SICAR (Structure Intracellular Concentration  
313 Activity Relationship) concept, is a step up to efficient drug designing. Briefly, SICAR  
314 connects the physicochemical drug properties to the efficacy of translocation through the  
315 bacterial membrane and the resulting intracellular accumulation. To achieve this goal, an  
316 extensive knowledge of the OM permeability control, including the contribution of sRNAs, is  
317 required.

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325

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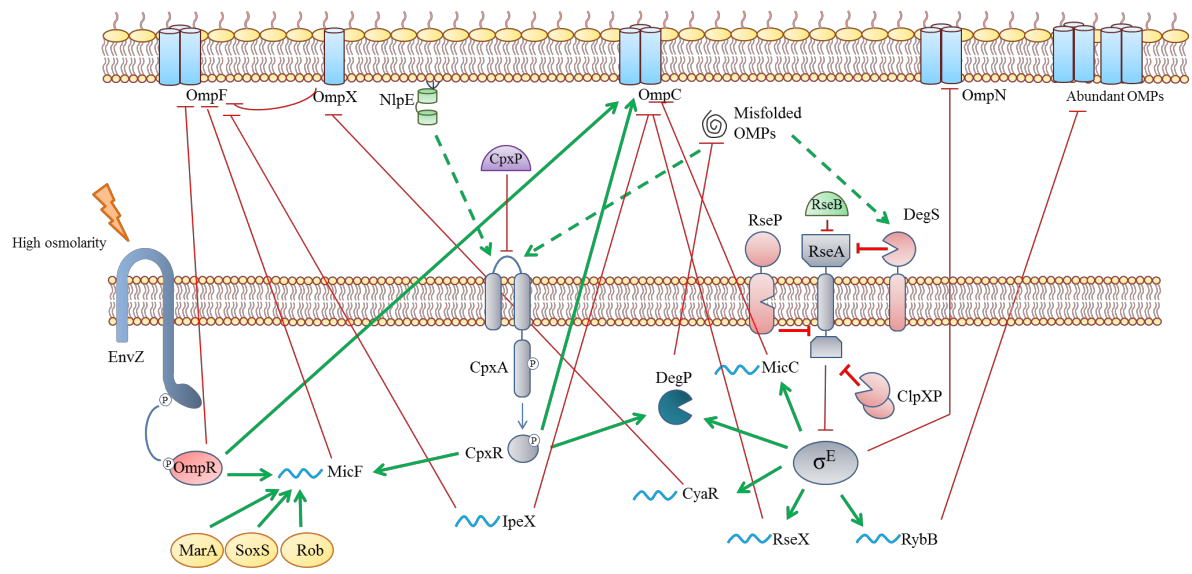
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608 **Key figure: Major regulatory pathways of porin regulation in *E. coli*:** EnvZ/OmpR [46],  
609 CpxAR and sigma E ( $\sigma^E$ ) [35] stress response systems are shown, along with known inducing  
610 cues and targets relevant to porin regulation. The upregulation is shown with thick green  
611 arrows, while the downregulation is shown with red lines. In the EnvZ/OmpR TCS,  
612 activation of the response regulator OmpR results in phosphorylated and OmpR~P  
613 downregulates the expression of OmpF both at the transcriptional and post-transcriptional  
614 levels, the latter via the MicF sRNA. The *mar-sox-rob* regulons also downregulate OmpF  
615 expression via MicF. Both the CpxAR and  $\sigma^E$  responses are activated by a variety of  
616 envelope stresses. For clarity, only periplasmic misfolded OMPs are represented here. On one  
617 hand, CpxR~P alters expression of multiple genes, including that of *micF*. On the other hand,  
618 the anti-sigma factor RseA is degraded by the successive action of two proteases, DegS and  
619 RseP at the periplasmic and the cytoplasmic site. Another protease, ClpXP specifically  
620 degrades the cytoplasmic RseA portion bound to  $\sigma^E$ , leading to its release. A number of  $\sigma^E$ -  
621 regulated sRNAs are indicated: MicC [78] downregulates OmpC and is coupled with *ompN*  
622 upregulation [80]; sRNA regulation of porins via CyaR [90], IpeX [91], RseX [86] and RybB  
623 [84, 88] are shown accompanied by their activators and porin targets; CyaR negatively  
624 regulates the expression of single channeled porin OmpX [30], which in turn negatively  
625 regulates the major porin OmpC. The details of all these interconnected pathways are  
626 discussed thoroughly in the text.  
627



628