Protection of the general stress response $\sigma S$ factor by the CrsR regulator allows a rapid and efficient adaptation of Shewanella oneidensis
Sophie Bouillet, Olivier Genest, Vincent Méjean, Chantal Iobbi-Nivol

To cite this version:
Sophie Bouillet, Olivier Genest, Vincent Méjean, Chantal Iobbi-Nivol. Protection of the general stress response $\sigma S$ factor by the CrsR regulator allows a rapid and efficient adaptation of Shewanella oneidensis. Journal of Biological Chemistry, American Society for Biochemistry and Molecular Biology, 2017, 292 (36), pp.14921-14928. 10.1074/jbc.M117.781443. hal-01696646

HAL Id: hal-01696646
https://hal.archives-ouvertes.fr/hal-01696646
Submitted on 25 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Protection of the General Stress Response $\sigma^5$ by CrsR Allows a Rapid and Efficient Adaptation of *Shewanella oneidensis*.

Sophie Bouillet, Olivier Genest, Vincent Méjean and Chantal Iobbi-Nivol
From Aix Marseille Univ, CNRS, BIP UMR7281, 13402 Marseille, France.

**Running title:** $\sigma^5$ protection in *S. oneidensis*

To whom correspondence should be addressed: Chantal Iobbi-Nivol, BIP-I MM, CNRS, 31, Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France, E-mail: iobbi@imm.cnrs.fr

**Keywords:** bacterial genetics; bacterial protein kinase; microbiology; stress response; bacterial anti-sigma factor

**Abstract**

To cope with environmental changes or stresses, bacteria develop various strategies and among them, the general stress response (GSR) is essential for survival. GSR is governed by an alternative transcriptional sigma factor named $\sigma^5$ (RpoS) that associates with the RNA polymerase and thus controls the expression of numerous genes. Previously, we have reported that posttranslational regulation of $\sigma^5$ in the aquatic bacterium *Shewanella oneidensis* involves the CrsR-CrsA partner-switching regulatory system, but the exact mechanism by which CrsR and CrsA control $\sigma^5$ activity is not completely unveiled. Here, using a translational gene fusion, we show that CrsR sequesters and protects $\sigma^5$ during the exponential growth phase and thus enables rapid gene activation by $\sigma^5$ as soon as the cells enter early stationary phase. We further demonstrate by an *in vitro* approach that this protection is mediated by the anti-sigma domain of CrsR. Structure-based alignments of CsrR orthologs and other anti-sigma factors identified a CsrR-specific region characteristic of a new family of anti-sigma factors. We found that CrsR is conserved in many aquatic proteobacteria and most of the time it is associated with CrsA. In conclusion, our results suggest that CsrR-mediated protection of $\sigma^5$ during exponential growth enables rapid adaptation of *S. oneidensis* to changing and stressful growth conditions and this ability is probably widespread among aquatic proteobacteria.

To cope with environmental changes or stresses, bacteria develop various strategies and among them, the general stress response (GSR) is essential for survival. GSR is governed by an alternative transcriptional sigma factor named $\sigma^5$ (RpoS) that associates with the RNA polymerase and thus controls the expression of numerous genes, for example its regulon contains more than 500 genes in *Escherichia coli*. As a consequence, $\sigma^5$ availability is tightly regulated at transcriptional, translational and posttranslational levels, leading to an increase of $\sigma^5$ in response to stresses or signals like, for instance, starvation or pH modifications, and conversely to a decrease of this sigma factor under favorable conditions (1, 2). In *E. coli*, the posttranslational regulation of $\sigma^5$ is driven by the ClpXP machinery in concert with the adaptor protein RssB. During exponential phase, RssB binds to $\sigma^5$ and addresses it to the protease complex (3). To counteract the role of RssB when $\sigma^5$ is required, the anti-adaptor proteins IraD, IraM and IraP interact with RssB.
and prevent the degradation of $\sigma^5$ (4). The posttranslational regulation of sigma B controlling the GSR has been extensively studied in the gram positive bacterium, Bacillus subtilis. Sigma B is posttranslationally regulated by the RsbWV partner-switching mechanism. RsbW is an anti-sigma factor that sequesters sigma B and phosphorylates RsbV when bacteria are under favorable conditions, and RsbV is an anti-sigma factor antagonist that binds RsbW and frees sigma B under stressful conditions (5, 6). In the latter case, dephosphorylation of RsbV is triggered by specific phosphatases (RsbU and RsbP). In a recent study, we have shown that in Shewanella oneidensis, a gram negative bacterium, $\sigma^5$ posttranslational regulation is also controlled by a partner-switching mechanism involving CrsR and CrsA (Fig. 1) (7). CrsR is a three-domain response regulator comprising a receiver domain (D1), a phosphatase domain (D2) and a kinase anti-sigma factor domain (D3), and CrsA is an anti-sigma factor antagonist. In the absence of signal, $\sigma^5$ is sequestered since it is bound to the anti-sigma factor domain D3 of CrsR that phosphorylates the anti-sigma factor antagonist CrsA (CrsA-P). When a stress arises, the phosphatase activity of $\text{CrsR}_{\text{D2}}$ dephosphorylates CrsA-P, CrsA can thus bind to $\text{CrsR}_{\text{D3}}$ and liberates $\sigma^5$ which can in turn interact with the RNA polymerase to allow the adaptation of bacteria to their environment. CrsR belongs to the GHKL ATPase/kinase superfamily that comprises proteins with little primary sequence homology aside from the conserved Bergerat motif (N-, G1-, G2-Boxes) and similar structural fold (8). Among its members, bacterial anti-sigma factor proteins or domains such as (9, 10) SpoIAB, $\text{CrsR}_{\text{D3}}$ or RsbW constitute a subfamily of kinases that presents the conserved Bergerat ATP-binding site and also a defined region of dimerization (9, 10). Moreover, the anti-sigma factor can be a protein per se (RsbW, SpoIAB in B. subtilis) or a domain of a more complex protein (the first domain of SyPE in Vibrio fisheri or the third domain of CrsR in S. oneidensis and HsbR in Pseudomonas aeruginosa) (7, 11–13).

In our previous study, we have unveiled the posttranslational regulation of S. oneidensis $\sigma^5$ by detailing the successive steps of the CrsR-CrsA partner-switching mechanism. Here, we show that this mechanism allows a rapid bacterial adaptation in versatile environments by protecting $\sigma^5$ from proteolysis and thus $\sigma^5$ remains available when necessary. In addition, we reveal that $\text{CrsR}_{\text{D3}}$ belongs to a new family of anti-sigma factor domains widespread in aquatic proteobacteria.

Results

Role of CrsR in the posttranslational regulation of $\sigma^5$. The question we posed was what happens to $\sigma^5$ when S. oneidensis is under favorable conditions? In a previous work, we have clearly identified the protein CrsR as a $\sigma^5$ anti-sigma factor. Indeed, it was shown that CrsR is bound to $\sigma^5$ when the bacterium is under favorable environment while under stressful conditions CrsR frees RpoS and binds the anti-sigma factor antagonist CrsA (Fig. 1). $\sigma^5$ can thus act as a transcriptional regulator for its regulon. $\sigma^5$ activity can be followed in vivo by using the $\text{dps-lacZ}$ fusion as previously shown (7). During exponential growth, the transcription level of the $\text{dps-lacZ}$ fusion is at a basal level while at stationary phase it increases drastically (Fig. 2). Moreover, we had observed that during exponential growth, the level of transcription of the fusion was lower in the absence of CrsR (strain ΔcrsR harboring $\text{dps-lacZ}$ fusion) than in its presence (strain WT harboring $\text{dps-lacZ}$ fusion). Thus, we wonder whether $\sigma^5$ could be protected by CrsR during the exponential growth of the bacterium in order to be quickly available in case a stress signal arises. To answer this question, an in vivo experiment measuring $\sigma^5$ activity was done. To this end, a $\text{dps-lacZ}$ chromosomal fusion, previously shown to be $\sigma^5$ dependent (7 and Fig. 2A), was introduced in a crsR-deleted strain (Fig. 2). As a control, the mutated strain was complemented by a chromosomal insertion of the wild type copy of $\text{crsR}$. As expected, during the exponential growth, a basal level of $\beta$-galactosidase activity was measured in the three strains with that of $\text{crsR}$-deleted strain as low as that of the rpoS mutant. At early
stationary phase (10 hours), the activity increased strongly under the control of σ^5 in the wild type and complemented strains, whereas in the ΔcrsR strain a significant increase of β-galactosidase activity was observed only at late stationary phase (Fig. 2A) with it reaching a plateau of lower value (Fig. 2A). This result indicates that in the absence of CrsR, σ^5 activity is delayed. In contrast, in the presence of CrsR, the adaptation of the bacteria is probably faster since the σ^5-dependent regulation is more rapidly effective. Moreover, when the crsR deletion was complemented, no time shift was detected and induction levels were similar to that of the wild type strain. It is noteworthy that the growth of the three strains was similar and that the delay in the activity was thus not correlated to the growth stage of the bacteria (Fig. 2B). This result is in favor of a protective role of CrsR towards σ^5 (Fig. 2A), and we therefore wanted to look at the level of σ^5 in the presence or the absence of CrsR.

Unfortunately, we were unable to detect σ^5 by western blot in the wild type strain during exponential phase. We thus decided to overproduce σ^5. For this purpose, σ^5 was produced from a plasmid introduced in wild type S. oneidensis (MR1), ΔcrsR and ΔcrsA strains. The crude extracts of the three exponentially grown strains were then submitted to SDS-PAGE and the presence of σ^5 was revealed by western blot. A band corresponding to σ^5 was observed for each strain but the amount of σ^5 are much higher for the wild type and ΔcrsA strains, than that obtained in the absence of CrsR (Fig. 3A). To confirm these results, we then tested the stability of σ^5 by an in vitro approach. Purified σ^5 was incubated with the crude extract of strain MR1 carrying either the control vector (ptac) or the pCrsR plasmid allowing the overproduction of CrsR, and σ^5 stability was followed as a function of time by western blot. We found that after 2 hours of incubation, the band corresponding to σ^5 almost disappeared when σ^5 was incubated with the control crude extract, whereas the intensity of the band was less reduced when σ^5 was incubated with the extract overproducing CrsR (Fig. 3B). Since in E. coli σ^5 degradation depends on the Clp machinerie, we tested whether in S. oneidensis, the Clp proteases are also involved in σ^5 stability. Purified σ^5 was incubated with the crude extract of MR1 or ΔclpP strains, and stability of σ^5 was followed as above (Fig. 3C). It appears that under these conditions, there is no differences in the pattern of degradation in the presence or in the absence of the ClpP protease. This suggests that another protease is involved in S. oneidensis σ^5 proteolysis. Altogether, these experiments confirm that CrsR protects σ^5 against degradation (Fig. 3).

CrsR and the CrsR-CrsA partner-switch are widespread in aquatic proteobacteria

To determine if the novel regulation of σ^5 we found in S. oneidensis could be conserved in other bacteria, we searched for CrsR-like proteins in bacterial genomes. This bioinformatics analysis revealed more than six hundreds CrsR homologs, all sharing the same domain organization (i. e. a receiver, a phosphatase domain and a kinase/anti-sigma factor domain). Strikingly, all of the CrsR homologs were found in Proteobacteria, with the exception of 5 homologs belonging to the Nitrospirae (one) and to the Deferribacteres (four). These CrsR homologs were mainly present in the γ-Proteobacteria class, although several representatives also appeared in the α-, β-, δ- and ε-Proteobacteria classes. Interestingly, no CrsR homolog was identified in the Enterobacteriales. Indeed, they were rather found in several other orders of the γ-Proteobacteria, three of them containing about 80% of the CrsR homologs (namely the Alteromonadales to which the Shewanellaceae belongs, the Pseudomonadales and the Vibrionales). A phylogenetic tree was then constructed using a subset of representative CrsR homologs (see Materials and Methods) (Fig. 4). The genetic environment (DNA length ≤ 20 kb) of the corresponding crsR genes was then analyzed seeking for crsA. We identified genes encoding CrsA homologs nearby the crsR genes in the majority of the bacterial genome analyzed (45 out of 59 CrsR homologs, Fig. 4). These results lead us to propose that the CrsR-CrsA partner-switching system is widespread among the Proteobacteria, and thus suggest that this mechanism of posttranslational...
regulation of σ is almost general in aquatic proteobacteria.

CrsR belongs to a new family of anti-sigma factor proteins

When searching for CrsR homologs in sequence data bank as we did for the phylogenic study, we did not hit the well-studied anti-sigma factors such as SpoIIAB, RsbW and SypE. An explanation can be that SpoIIAB and RsbW are organized as a single domain and the organization of the three domains of SypE is different from that of CrsR. In fact, analysis of the sequence alignment of CrsR03 with these three anti-sigma factors and CrsR03 homologs obtained from the phylogenic tree highlights an additional region present in CrsR03. Interestingly, this region is conserved in anti-sigma factor domains of CrsR homologs present in figure 4 (Fig. 5A). This region stretches from Leu469 to Ser496 (S. oneidensis CrsR numbering) between the N- and G1-Boxes which are conserved motifs of the GHKL ATPase/kinase superfamily (Fig. 5A). On the basis of solved structures of anti-sigma factor proteins, the predictive model of the 3D-structure of CrsR03 was designed using I-Tasser program (14). The structural organization of the stretch of 28 amino acids described above was simulated as an unfolded loop (from Leu469 to Asp488) followed by a short alpha helix (from Ser489 to Arg493) at the surface of the protein (Fig. 5B). This additional region defines a new class of anti-sigma factors.

Discussion

We have recently shown that σS is regulated by a partner switch in S. oneidensis (7). The third domain of CrsR (CrsR03) is an anti-σ factor domain that sequesters σS in the absence of stress. In starvation condition (stationary phase), σS is released from CrsR due to the binding of the anti-σ factor antagonist CrsA to CrsR03. Phosphorylation or dephosphorylation of CrsA results from the action of either the kinase of CrsR03 or the phosphatase of CrsR02, respectively (Fig. 1). In the absence of stress, CrsR02 is inactive while CrsA is phosphorylated by CrsR03 and CrsA-P cannot bind CrsR.

In this study, we show that CrsR protects σS against proteolysis under no stress condition. We also observed that σS induced dps during the early stationary phase (Fig. 2) and the induction level remains constant from early to late stationary phases. This result strongly suggests that the entire pool of σS is released from CrsR when cells enter into the stationary phase. It will be interesting to confirm this possible on/off mechanism using other σS-dependent genes and various stress, although transcriptional regulation could partially contribute to σS regulon induction. Another striking point is that dps is also induced in a ΔcrsR mutant but the induction level increased slightly during the stationary phase and it did not reach that of the wild type strain. We thus propose that an additional regulatory mechanism operates during the stationary phase, possibly by inactivating the protease targeting σS or by protecting σS with a specific escort protein produced during the stationary phase. In contrast to σS of E. coli, σS of S. oneidensis is not degraded by the Clp protease in our experimental condition. This data confirms that the post-translational regulations of σS of E. coli and S. oneidensis have no similarity. In E. coli, σS is degraded in the absence of stress. Therefore, σS must be synthesized de novo during stressful conditions and the response is thus delayed reaching its maximum during the late stationary phase (1, 2, 15). In S. oneidensis, σS is always available and can activate quickly the target genes in the presence of a stress, allowing an efficient cell adaptation. We suppose that, when the stress disappears, CrsR could again sequester σS. If true, this partner switch allows a rapid and reversible answer with a low energy cost.

Interestingly, the protective role of an anti-sigma factor was previously described for σ7 in the gram positive Streptomyces coelicolor (16). The preservation of the sigma factor even when no signal is present could be an efficient way to adapt for bacteria living in versatile biotopes. A similar effect was also observed for
the flagellar sigma factor FlIA, which is protected by the anti-sigma factor FlgM (17).

Using a bioinformatics approach, we identified a large family of proteobacterial proteins homologous to CrsR of *S. oneidensis*. It is striking that among the analyzed bacteria including, *Shewanella* sp., *Pseudomonas* sp. and *Vibrio* sp., many live in aquatic environments and have to deal with a wider range of stress than *E. coli* and other enterobacteria that live in more restricted habitats. In addition, the CrsR-CrsA partner-switch homologs could be involved in the regulation of other alternative σ factors. Indeed, although Alphaproteobacteria do not possess a σ^D^ homolog, but instead a sigma σ^EcfG^ factor, CrsR-CrsA partner-switch is conserved in some of them (18–20). For example, *Magnetococcus marinus* MC1 does not encode the NepR-PhyR proteins that usually regulate σ^EcfG^, but possesses *crsA* and *crsR* homologs (Fig. 4). Taken together, these data suggest that the CrsR-CrsA partner-switch is a widespread regulatory system involved in the posttranslational regulation of GSR sigma factors.

Finally, we identified in this study a region of the D3 domain specific of anti-sigma factors of the CrsR family. It is a loop comprised between the N- and G1 conserved boxes found in kinase sequences (Fig. 5A). This loop comprising 28 amino acid residues is characteristic of CrsR homologs presenting the same three-domain organization and thus could be the trademark of a new family of anti-sigma factors found in various classes of the phylum of proteobacteria (Fig. 4). The structure prediction of this region suggests no particular fold while the rest of the domain is reminiscent of that observed in NepR of *Alphaproteobacteria* although, even NepR and CrsR are both anti-sigma factor proteins, they are not related. The disordered region of NepR was shown to participate in the binding of its substrates (PhyR and σ^EcfG^) (19, 21). It would be interesting to determine if the additional extension is also involved in the binding of CrsR partners. Unfortunately, so far any modification of this region leads to instable variants allowing no conclusion about the role of this extra region.

In conclusion, this study demonstrates the role of CrsR towards σ^D^ during the exponential phase of the bacterial growth. Indeed, it is now clear that in the absence of stress, the interaction between the two proteins leads to a protection of σ^D^. This latter can be released from CrsR as soon as CrsA is dephosphorylated when an environmental stress signal is detected by an yet unknown signal transduction pathway. This mechanism, which is highly conserved among proteobacteria, could allow a faster adaptation of the bacteria under versatile conditions.

**Experimental procedures**

*Medium, growth conditions, strains, and plasmids* - Strains were routinely grown in LB medium at 28°C and 37°C for *S. oneidensis* and *E. coli*, respectively (22). When appropriated, antibiotics were used at the following concentrations: kanamycin (25 μg/mL), streptomycin (100 μg/mL), and chloramphenicol (25 μg/mL). All *S. oneidensis* strains used in this study (WT, ΔlbpP, WT *dps-lacZ* fusion, ΔrpoS *dps-lacZ*, ΔcrsR *dps-lacZ* fusion and ΔcrsR/crsR *dps-lacZ* fusion) are derivatives of the MR1-R strain referred as WT (7, 23). Complementation in trans of SO2119 (*crsR*) named ΔcrsR/crsR was done by cloning two 500 bp fragments containing *Xmal* and *Xhol* restriction sites and flanking the site of insertion (between the genes SO2126 and SO2127). The fragment was cloned into the pKNG101 suicide vector (24) at the *SalI* and *Spel* restriction sites as described before (25). The coding sequence of *crsR* (SO2119) was then cloned in frame after a consensus σ^70^ promoter sequence (TTGACAn_{17}TATAAT) and a consensus RBS sequence (AGGAGA) into modified pKNG101 and was introduced into *E. coli* CC118λpir and then transferred to ΔcrsR as described before for deletion mutants (7). The pKNG101 vector containing the *dps-lacZ* fusion was then transferred to ΔcrsR/crsR strain by conjugation as previously described (7).
The plasmids used in this study are the followings. pBrpoS corresponds to the pBAD33 vector carrying rpoS (SO3432) coding sequence in frame with N-terminal StrepTagII sequence, pETσ5-52 vector corresponds to the pET-52b vector carrying rpoS (SO3432) sequence (7). pTCrsR vector corresponds to the p33Tac vector (pBAD33 derivative vector with ara promoter replaced by lac promoter) carrying crsR coding sequence (SO2119).

Expression and purification of recombinant σ5 protein- Recombinant protein Strep-σ5 was produced and purified from E. coli BL21(DE3) strain containing the plasmid pETσ5-52 as described before (7).

In vivo assays- To follow the activity of the dps-lacZ fusion in stationary phase, the strains were grown at 28 °C anaerobically in LB medium supplemented with trimethylamine oxide (TMAO, 10 mM) as final electron acceptor (26). Samples of cultures were collected at different times and β-galactosidase activities were measured in Miller units as previously described (22).

In vitro degradation systems- CrsR, protein was produced from MR1-R strains containing the plasmids p33Tac and pTCrsR. At OD600=0.4, IPTG (1 mM) was added to overproduce the protein. Cells were then grown for 2 additional hours, collected by centrifugation, washed with Tris-HCl pH 7.6 buffer and lysed by adding 1:10 PopCulture® reagent (Novagen®) and lysozyme (1mg/mL final concentration). The crude extracts were collected by centrifugation at 13000 rpm for 15 minutes (27). MR1-R and ΔclpP strains were harvested during exponential growth and crude extract were prepared as above. Crude extracts were then diluted at 5 mg/mL of total proteins in Tris-HCl pH 7.6 buffer and reactions were started by adding 0.5 μM of σ5 protein. Samples were incubated at 25°C and aliquots were collected at times 0 and 2 hours or 0, 0.5, 1 and 2 hours. Loading buffer was added and samples were heated for 5 minutes at 95°C before migration by electrophoresis with a Bolt™ 4-12% Bis-Tris gel (Invitrogen). Proteins were then visualized after western blot using Strep-Tactin probe HRP conjugated antibody (IBA).

In vivo production of σ5 protein- σ5 protein was produced from MR1-R, ΔcrsR and ΔcrsA strains containing the plasmid pBRpoS. Cells were grown for 1 hour before 0.02% arabinose was added and cells were incubated for 2 hours under shaking. The crude extracts were prepared and treated as described above. σ5 was visualized after western blot using anti-σ5 rabbit antibody (gift from Susan Gottesman), followed by anti-rabbit HRP-conjugated antibody (Sigma Aldrich).

Bioinformatics analyses- The proteins sharing homologies with CrsR were found in the NCBI non-redundant protein sequences database using the protein BLAST search tool. For the phylogenetic tree construction, the searches were made independently on the different classes of Proteobacteria (α-, β-, γ-, δ-, ε-Proteobacteria) as well as on bacteria, with the exclusion of the Proteobacteria. For the γ-Proteobacteria, the searches were made separately on each order composing this class. One representative sequence for each genus was subsequently selected, except for Shewanella, Vibrio and Pseudomonas. We chose the proteins sharing the highest E-value with CrsR on the whole length of the proteins. For the phylogenetic analysis, we used the “Phylogeny.fr” software in the “one-click” mode, i.e. with the default parameters optimized by the authors (28) (http://www.phylogeny.fr/). The main steps performed by this software correspond to multiple alignments of the CrsR homologs using the MUSCLE version 3.8.31 method, alignment curation by GBLOCKS version 0.9b and phylogeny using PhyML version 3.1 method using 100 bootstrap replicates. For the tree rendering step, we used the software “FigTree” version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) in which we entered the result in Netwick format obtained with “Phylogeny”. After the first phylogenetic analysis, we manually removed the unnecessary sequences and a second phylogenetic analysis was performed. The neighborhood of the genes coding for the CrsR homologs was extracted from the databases.
using NCBI, MAGE (https://www.genoscope.cns.fr/agc/microscope/home/) and KEGG (http://www.genome.jp/).

Sequences alignment and tertiary structure prediction- Representative sequences of CrsR from different classes, orders and genus were selected. HATPase domains from these proteins and SypE (from Vibrio fisheri), RsbW and SpoIIAB (from Bacillus subtilis) were aligned using the Clustal Omega program (EMBL) and the highlighted and conserved amino acid residues were generated using the BoxShade (ExPASy) server. The secondary structure of CrsR\textsubscript{D3} was predicted by using the PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/). Structure of CrsR\textsubscript{D3} was predicted by using I-Tasser server and the model having the highest C-score (-1.14) was annotated and shown in Fig. 5B (14) (http://zhanglab.Ccmb.med.umich.edu/I-TASSER/).

Acknowledgements

We would like to thank Susan Gottesman and Aurélia Battesti for the gift of antibodies and strains, members of the group and in particular Olivier Lemaire for fruitful discussions, Ahmed Zellat for technical assistance and Kevin Copp for reviewing the manuscript. This work was supported by the Centre National de la Recherche Scientifique and Aix-Marseille Université (AMU). S.B. was supported by a Ministère de l’Enseignement Supérieur et de la Recherche fellowship and AMU.

Conflict of interest: The authors declare that they have no conflict of interest with the contents of this article.

Author contributions: All authors were involved in conduction of experiments, analysis of the results, and the writing of the paper.

References

1. Hengge, R. (2011) The General Stress Response in Gram-negative Bacteria. in Bacterial Stress Responses, 2nd edition, American Society for Microbiology (ASM), 2nd Ed., p. 251, Storz, Gisela and Hengge, Regine, Chapter 15, 251
2. Battesti, A., Majdalani, N., and Gottesman, S. (2011) The RpoS-mediated general stress response in Escherichia coli. Annu. Rev. Microbiol. 65, 189–213
3. Hengge, R. (2009) Proteolysis of sigmaS (RpoS) and the general stress response in Escherichia coli. Res. Microbiol. 160, 667–676
4. Battesti, A., Hoskins, J. R., Tong, S., Milanesio, P., Mann, J. M., Kravats, A., Tsegaye, Y. M., Bougdour, A., Wickner, S., and Gottesman, S. (2013) Anti-adaptors provide multiple modes for regulation of the RssB adaptor protein. Genes Dev. 27, 2722–2735
5. Dufour, A., and Haldenwang, W. G. (1994) Interactions between a Bacillus subtilis anti-sigma factor (RsbW) and its antagonist (RsbV). J. Bacteriol. 176, 1813–1820
6. Price, C. W. (2011) The general stress response in Bacillus subtilis and related Gram-positive bacteria. in Bacterial Stress Responses, 2nd edition, American Society of Microbiology (ASM), p. 301, Hengge Regine, Storz Gisela
7. Bouillet, S., Genest, O., Jourlin-Castelli, C., Fons, M., Méjean, V., and Iobbi-Nivol, C. (2016) The General Stress Response σ\textsubscript{S} Is Regulated by a Partner Switch in the Gram-negative Bacterium Shewanella oneidensis. J. Biol. Chem. 291, 26151–26163
8. Dutta, R., and Inouye, M. (2000) GHKL, an emergent ATPase/kinase superfamily. Trends Biochem. Sci. 25, 24–28

σ\textsubscript{S} protection in S. oneidensis
9. Campbell, E. A., Masuda, S., Sun, J. L., Muzzin, O., Olson, C. A., Wang, S., and Darst, S. A. (2002) Crystal Structure of the Bacillus stearothermophilus Anti-σ Factor SpoIIAB with the Sporulation σ Factor of. *Cell.* **108**, 795–807.

10. Masuda, S., Murakami, K. S., Wang, S., Anders Olson, C., Donigian, J., Leon, F., Darst, S. A., and Campbell, E. A. (2004) Crystal Structures of the ADP and ATP Bound Forms of the Bacillus Anti-σ Factor SpoIIAB in Complex with the Anti-anti-σ SpoIIA. *J. Mol. Biol.* **340**, 941–956.

11. Morris, A. R., and Visick, K. L. (2013) The response regulator SypE controls biofilm formation and colonization through phosphorylation of the syp-encoded regulator SypA in Vibrio fischeri. *Mol. Microbiol.* **87**, 509–525.

12. Houot, L., Fanni, A., de Bentzmann, S., and Bordi, C. (2012) A bacterial two-hybrid genome fragment library for deciphering regulatory networks of the opportunistic pathogen Pseudomonas aeruginosa. *Microbiol. Read. Engl.* **158**, 1964–1971.

13. Bhuwan, M., Lee, H.-J., Peng, H.-L., and Chang, H.-Y. (2012) Histidine-containing phosphotransfer protein B (HptB) regulates swarming motility through partner-switching system in Pseudomonas aeruginosa PA01 strain. *J. Biol. Chem.* **287**, 1903–1914.

14. Roy, A., Kucukural, A., and Zhang, Y. (2010) I-TASSER: a unified platform for automated protein structure and function prediction. *Nat. Protoc.* **5**, 725–738.

15. Becker, G., Klauck, E., and Hengge-Aronis, R. (2000) The response regulator RssB, a recognition factor for sigmaS proteolysis in Escherichia coli, can act like an anti-sigmaS factor. *Mol. Microbiol.* **35**, 657–666.

16. Mao, X.-M., Ren, N.-N., Sun, N., Wang, F., Zhou, R.-C., Tang, Y., and Li, Y.-Q. (2014) Proteasome involvement in a complex cascade mediating SigT degradation during differentiation of Streptomyces coelicolor. *FEBS Lett.* **588**, 608–613.

17. Barembruch, C., and Hengge, R. (2007) Cellular levels and activity of the flagellar sigma factor FlIA of Escherichia coli are controlled by FlgM-modulated proteolysis. *Mol. Microbiol.* **65**, 76–89.

18. Österberg, S., del Peso-Santos, T., and Shingler, V. (2011) Regulation of alternative sigma factor use. *Annu. Rev. Microbiol.* **65**, 37–55.

19. Fiebig, A., Herrou, J., Willett, J., and Crosson, S. (2015) General Stress Signaling in the Alphaproteobacteria. *Annu. Rev. Genet.* **49**, null.

20. Francez-Charlot, A., Kaczmarczyk, A., Fischer, H.-M., and Vorholt, J. A. (2015) The general stress response in Alphaproteobacteria. *Trends Microbiol.* **23**, 164–171.

21. Herrou, J., Willett, J. W., and Crosson, S. (2015) Structured and Dynamic Disordered Domains Regulate the Activity of a Multifunctional Anti-σ Factor. *mBio* **6**, e00910-15.

22. Miller, J. H. (1972) Experiments in Molecular Genetics. *Cold Spring Harb. Lab. Press*.

23. Bordi, C., Iobbi-Nivol, C., Méjean, V., and Patte, J.-C. (2003) Effects of ISSo2 insertions in structural and regulatory genes of the trimethylamine oxide reductase of Shewanella oneidensis. *J. Bacteriol.* **185**, 2042–2045.

24. Herrero, M., de Lorenzo, V., and Timmis, K. N. (1990) Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. *J. Bacteriol.* **172**, 6557–6567.

25. Baraquet, C., Théraulaz, L., Iobbi-Nivol, C., Méjean, V., and Jourlin-Castelli, C. (2009) Unexpected chemoreceptors mediate energy taxis towards electron acceptors in Shewanella oneidensis. *Mol. Microbiol.* **73**, 278–290.

26. Lemaire, O. N., Honoré, F. A., Jourlin-Castelli, C., Méjean, V., Fons, M., and Iobbi-Nivol, C. (2016) Efficient respiration on TMAO requires TorD and TorE auxiliary proteins in Shewanella oneidensis. *Res. Microbiol.* **10.1016/j.resmic.2016.05.004**.

27. Thibodeau, S. A., Fang, R., and Joung, J. K. (2004) High-throughput beta-galactosidase assay for bacterial cell-based reporter systems. *BioTechniques.* **36**, 410–415.

28. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M., and Gascuel, O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* **36**, W465–469.
**Figure legends**

**Figure 1. Model of the partner-switching mechanism involved in the regulation of σ^5 in S. oneidensis**

**A.** Under favorable conditions, the third domain of CrsR (D3) phosphorylates CrsA (CrsA-P), hampering the interaction between the two proteins and leading to the sequestration of σ^5 by the anti-sigma factor domain (D3) of CrsR. Thus, σ^5 is unable to promote the transcription of the genes from its regulon.

**B.** Under stress conditions, CrsR dephosphorylates CrsA-P via its phosphatase domain (D2). The anti-sigma factor antagonist CrsA then interacts with the anti-sigma factor domain of CrsR, driving the release of σ^5. σ^5 can thus bind the core of the RNA polymerase (Core RNA-Pol) and promotes the transcription of the genes involved in GSR, including the dps gene. D1 represents the receiver domain of CrsR and P the phosphoryl group.

**Figure 2. Effects of CrsR on σ^5 in vivo.**

**A.** The absence of CrsR delays the σ^5-dependent dps induction in stationary phase. Strains WT, ΔrpoS, ΔcrsR and ΔcrsR/crsR harboring the dps-lacZ fusion were grown until stationary phase, anaerobically with TMAO. β-galactosidase activities were measured at different times.

**B.** Growth of WT, ΔrpoS, ΔcrsR and ΔcrsR/crsR strains is similar. Curves represent the OD_{600} of strains used in A as a function of time.

For A and B, averages and SD from three independent experiments are shown.

**Figure 3. CrsR protects σ^5 from degradation in vivo and in vitro.**

**A.** σ^5 is protected from degradation by CrsR in vivo during exponential phase. Strains WT, ΔcrsA and ΔcrsR carrying pBRpoS were grown until exponential phase aerobically with 0.02% arabinose to induce σ^5 production. Crude extracts were submitted to SDS-PAGE and σ^5 was revealed by western blot with σ^5 antibodies.

**B.** σ^5 is protected in vitro by CrsR. Crude extracts of MR1 cells harvested during exponential phase and overproducing CrsR were incubated with purified Strep-σ^5 protein. Samples were collected at t_0 and t_2h, submitted to SDS-PAGE and σ^5 was detected by a StrepTactin antibody.

**C.** Stability of σ^5 in the absence of ClpP protease. Same experiment as above except that crude extracts were prepared from MR1 and ΔclpP cells. Collection time of the samples are indicated on the figure.

**Figure 4. Occurrence of CrsR homologs in Bacteria.**

Searches for homologous proteins to S. oneidensis CrsR were done using the bioinformatics BLAST tool and the sequences were assembled using the program “Phylogeny”. Bacteria are: Alpha-proteobacteria, Beta-proteobacteria, Delta-proteobacteria, Epsilon-proteobacteria, Deferribacteres (eubacteria except Proteobacteria), Gamma-proteobacteria (Alteromonadales, Chromatiales, Methylococcales, Oceanospirillales, Pseudomonadales, Thiocichales, Vibrionales). The symbol * indicates the presence of a homolog of the S. oneidensis gene crsA in the vicinity (≤ 20 kb) of the crsR homolog in the tied species. The symbol † indicates a genus. Among the genus Pseudomonas, the species P. aeruginosa, P. putida, P. chlororaphis, P. fluorescens, P. syringae, P. stutzeri were selected. The genus Vibrio includes V. mimicus, V. cholerae, V. vulnificus, and the genus Shewanella includes S.
Protection in *S. oneidensis*

*xiamenensis*, *S. decolorationis*, *S. sp. HN-41*, *S. baltica OS185*, *S. sp. ANA-3*, *S. sp. MR-7*, *S. putrefaciens* and *S. oneidensis MR-1*.

**Figure 5.** The GHKL ATPase/kinase domain of CrsR possesses an additional region.

A. Sequence alignment of the third domain of CrsR (CrsR\(_{D3}\)) from amino acids 447 to 527 of *S. oneidensis* and CrsR\(_{D3}\) homologs from γ-proteobacteria *Methylomonas denitrificans*, *Hydrogenovibrio marinus*, *Oceanospirillum beijerinckii*, *Halorhodospira halophila SL1*, *Glaciecola chathamensis*, *Vibrio cholerae N16961*, *Shewanella baltica OS185*, *Shewanella decolorationis* and β-proteobacterium *Leeia oryzae*, with the anti-σ factors RsbW and SpoIIAB of *B. subtilis* (Firmicutes), and the anti-σ domain of SypE (from *V. fisheri*, γ-proteobacterium). The conserved ATP binding Bergerat-fold N- and G1- boxes are indicated. The additional region is framed in red and the secondary structure prediction of CrsR\(_{D3}\) is drawn above the alignment. Conserved residues in the additional region are in blue.

B. Comparison of the tertiary structure of SpoIIAB from *Bacillus subtilis* (9, 10) and the predicted structure of CrsR\(_{D3}\). Helix are in green, sheets in pink, loops in orange and the extra region in yellow and blue, and zoomed in the circle. The highly conserved residues in the extra region appear in blue and are annotated in the zoomed box. CrsR\(_{D3}\) structure was predicted using I-Tasser program.
Figure 1
Figure 2
Figure 3
Figure 4
### A. Consensus Sequence

| Species                        | Sequence |
|-------------------------------|----------|
| S. oneidensis                 | ATNFCR... |
| S. baltica                    | ATNFCR... |
| S. decolorationis             | ATNFCR... |
| Methylomonas                  | LQNYFL... |
| Glaciecola                    | NTLFDV... |
| Hydrogenovibrio               | LISFKED... |
| Vibrio cholerae               | GGDLDL... |
| Halorhodospira                | LEEDR... |
| Oceanospirillum               | VESNKL... |
| Leelia oryzae                 | TRQRSE... |
| SypE                          | NTTVL... |
| SpoIIAB                       | TMDELT... |
| RebW                          | TYDEIE... |
| **Consensus**                 | qvfmmva... |

### B. Structure Diagrams

- **SpolIIAB**
- **CrsR_{D3}**

---

**Figure 5**