Structural basis for sigma factor mimicry in the general stress response of Alphaproteobacteria

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**AUTHOR SUMMARY**

In all organisms, expression of distinct sets of genes is crucial to ensure their development and to allow them to adapt to environmental conditions. In bacteria, sigma factors, which are subunits of RNA polymerase, play a central role in these processes by deciphering which gene products are made at a given time. Sigma factors are controlled, in turn, by sequestration by inhibitors. To ensure correct and timely reprogramming of cells, sigma factor availability is subject to regulatory cues. Here, we show at the atomic level how a recently discovered sigma factor is freed from its inhibitor as follows: upon sensing an environmental cue, an inhibitor antagonist exposes an otherwise hidden surface that mimics a sigma factor, which then binds to and inactivates the inhibitor.

The system studied here governs the general stress response of the Alphaproteobacteria class (1), which encompasses a large number of widely dispersed and diverse species. The central players in the system that regulates the general stress response are the sigma factor \( \sigma_{EcfG} \), the anti-sigma factor NepR, and the anti-sigma factor antagonist PhyR. PhyR is a response regulator, which is activated upon phosphorylation at its so-called “receiver domain” (PhyR\(_{REC}\)). It is intriguing that it harbors an effector domain (sigma factor-like output domain, PhyR\(_{SL}\)), which mimics a sigma factor. In the current model depicted in Fig. P1, PhyR\(_{REC}\) inhibits the antagonistic function of PhyR\(_{SL}\) in unphosphorylated PhyR; PhyR\(_{REC}\) phosphorylation turns off this inhibition, allowing NepR to bind PhyR\(_{SL}\) (2). In agreement with this model, the crystal structure of unphosphorylated PhyR shows extensive contacts between these two domains (3). However, how phosphorylation of PhyR allows it to mimic the true sigma factor \( \sigma_{EcfG} \) and to sequester NepR remains unknown.

To address this question, the structure of PhyR\(_{SL}\) bound to NepR was solved using NMR. The NMR structure shows that a discrete region of NepR, which is very similar in all members of Alphaproteobacteria, folds upon binding to PhyR\(_{SL}\) into two helical segments that extend along the surface of PhyR\(_{SL}\). Mutations that change the amino acid sequences in these segments disrupt binding.

To explore the structural basis of sigma factor mimicry, we determined whether NepR binds to the true sigma factor \( \sigma_{EcfG} \) in a similar way as to PhyR\(_{SL}\). A homology model of the \( \sigma_{EcfG} \)-NepR complex was built based on the NMR structure of the PhyR\(_{SL}\)-NepR complex. In line with NMR data, suggesting that NepR adopts a similar overall structure in both complexes, monitoring the strength of interactions of mutant proteins demonstrated the equal importance of hydrophobic amino acids of NepR for PhyR\(_{SL}\) and \( \sigma_{EcfG} \) binding, as well as the involvement of the same sequences of PhyR\(_{SL}\) and \( \sigma_{EcfG} \) for contacting NepR. However, polar amino acids involved in PhyR\(_{SL}\) binding are absent in the \( \sigma_{EcfG} \)-NepR complex. This and other structural observations likely account for the tighter binding of NepR to PhyR compared with \( \sigma_{EcfG} \). On the basis of these experimental findings and from the fact that PhyR\(_{SL}\) and \( \sigma_{EcfG} \) are present at similar levels in cells, we conclude that this difference in binding accounts for the partner switch when bacteria are stressed under natural conditions.

The structure of the PhyR\(_{SL}\)-NepR complex presented here, together with our comparative functional analyses, demonstrates the structural basis for sigma factor mimicry that governs the general stress response of Alphaproteobacteria. This discovery should stimulate future research on the role of molecular mimicry on transcription regulation in prokaryotes.


The authors declare no conflict of interest.

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Data deposition: The NMR atomic coordinates reported in this paper have been deposited in the Protein Data Bank, [wwwpdb.org](http://www.pdb.org) (PDB ID code 2LFW) and NMR chemical shifts and restraints have been deposited in the BioMagResBank, [www.bmrb.wisc.edu](http://www.bmrb.wisc.edu) (accession no. 17784).

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