

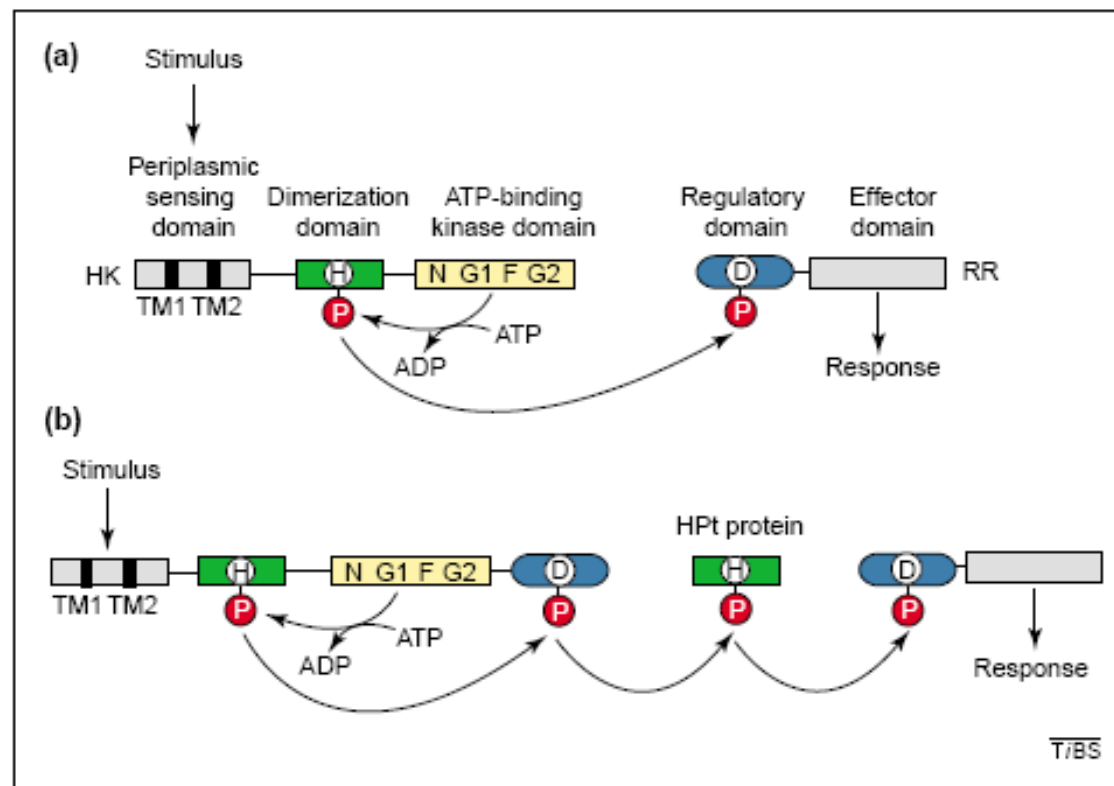
Sigma factor mimicry involved in regulation of general stress response

Anne Francez-Charlot¹, Julia Frunzke¹, Christian Reichen, Judith Zingg Ebnetter, Benjamin Gourion, and Julia A. Vorholt²

Institute of Microbiology, Eidgenössische Technische Hochschule Zurich, 8093 Zurich, Switzerland

Ahmet Bozdog
Feb 15th, 2010

Bacterial Responses to Environmental Stresses-The two component system



Bacterial Response to Environmental Stresses- The Extracytoplasmic Function (ECF) Sigma Factors

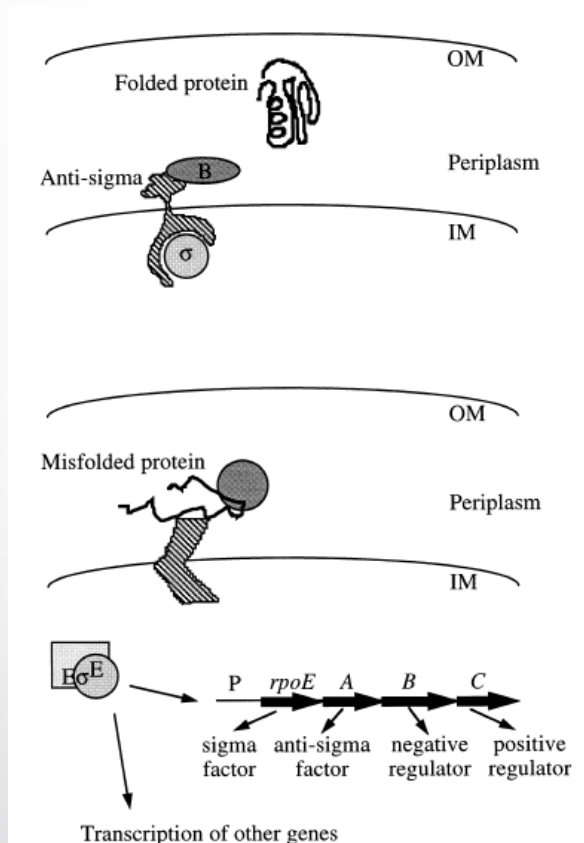


Table 3. Inducers of the σ^E response.

Overexpression inducers (multicopy)

Protein name	Protein location	Cause of increased σ^E activity
AsnB	Periplasm	Truncated and unstable
OmpX	Outer membrane	Imbalanced synthesis
OmpK	Outer membrane	Imbalanced synthesis, (unpublished)
DsbD	Inner membrane	Misfolding of disulphide-containing proteins
PBP2	Peptidoglycan	Imbalanced synthesis

Loss of function inducers

Gene name	Protein location	Protein function
<i>dsbA</i>	Periplasmic	Thiol:disulphide oxidant
<i>dsbB</i>	Inner membrane	Oxidizes DsbA
<i>dsbC</i>	Periplasmic	Thiol:disulphide isomerase
<i>surA</i>	Periplasmic	Peptidyl prolyl isomerase
<i>fkpA</i>	Periplasmic	Peptidyl prolyl isomerase
<i>skp</i>	Periplasmic	Assisting OMPs folding/insertion in outer-membrane
<i>rseB</i>	Periplasmic	Putative chaperone, signalling molecule
<i>ompK</i>	Outer membrane	Biogenesis of outer membrane (unpublished)
<i>htrM</i>	Cytoplasmic	Epimerase of LPS biogenesis

Bacterial Response to Environmental Stresses- The Extracytoplasmic Function (ECF) Sigma Factors

Table 1. Consensus sequences of σ^E -dependent promoters.

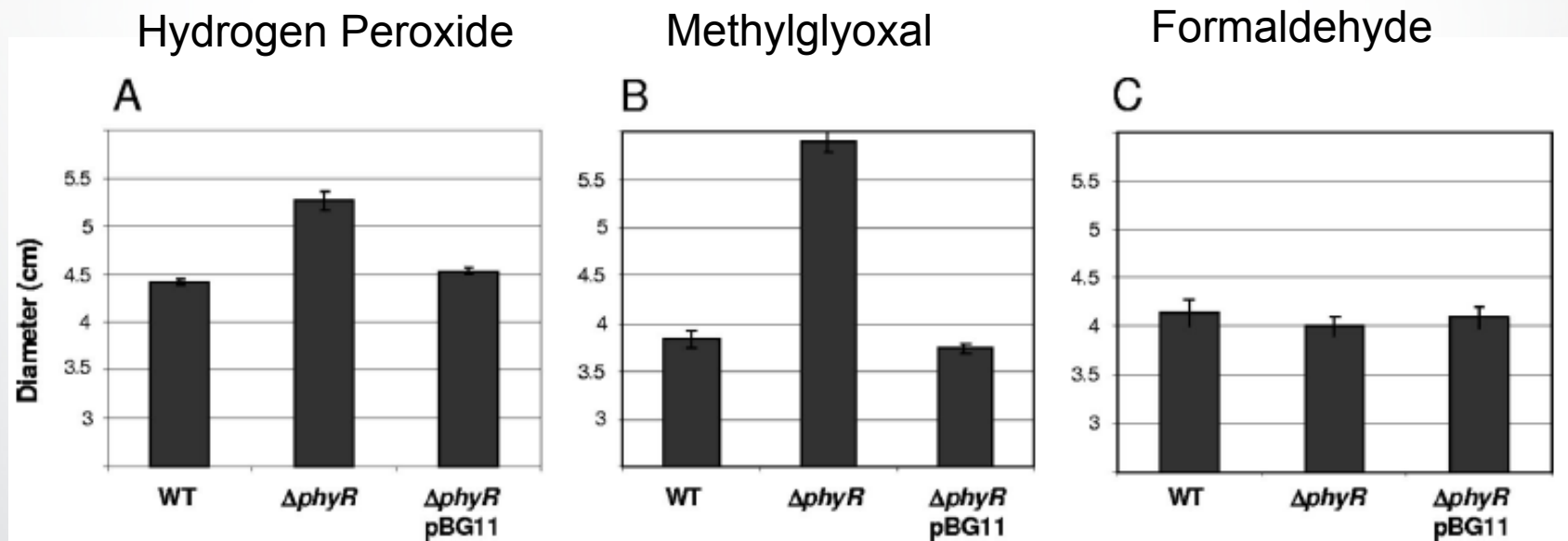
	-35 box	nt spacing	-10 box
<i>E. coli</i>			
rpoHP3	GAACTT	16 bp	TCTGA
htrA	GAACTT	16 bp	TCTGA
rpoEP2	GAACTT	16 bp	TCTGA
fkpAP2	AAACTA	16 bp	TCTGA
ompKP2	GAATTT	16 bp	TCTTA
<i>P. aeruginosa</i>			
rpoHP3	GAACTT	16 bp	TCAGA
algU	GAACTT	16 bp	TCTAT
algD	GAACTT	16 bp	TCCTA
<i>S. coelicolor</i>			
dagAP2	GAACTT	16 bp	CTCTC
<i>S. antibioticus</i>			
phsA	GAACGC	16 bp	GTCTC
<i>M. xanthus</i>			
carQRS	GAAACA	16 bp	GTAGA
<i>B. subtilis</i>			
sigX(b)	TAACTT	16 bp	TACGA

Missiakas and Raina, 2010

PhyR is Involved in the General Stress Response of *Methylobacterium Extorquens* AM1

- PhyR (Phyllosphere-induced regulator) has domains of both systems; an amino terminal ECF sigma factor-like domain and a carboxy terminal receiver domain of response regulator.
- PhyR has a large regulon, 246 targets, most of which are positively controlled.
- An ECF-type promoter was identified upstream of one-third target genes, *in silico*.
- Among the target genes are stress response genes like *katE*, *osmC*, *htrA*, *gloA*, *dps* and *uvrA*

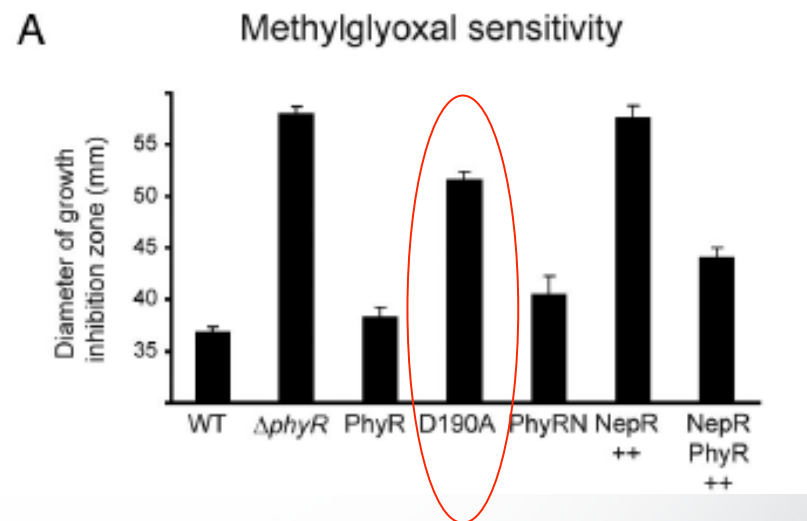
PhyR is Involved in the General Stress Response of *Methylobacterium Extorquens* AM1



Disk Diffusion Assays

Characterization of PhyR as a Response Regulator

- Five possible phosphorylation sites in receiver domain: Glu-144, Asp-145, Asp-190, Thr-218, and Lys-238, with Asp-190 as predicted phosphorylation site
- Asp-190 was substituted with Alanine



Phosphorylation of PhyR is required to activate the protein and Asp-190 is the site of phosphorylation

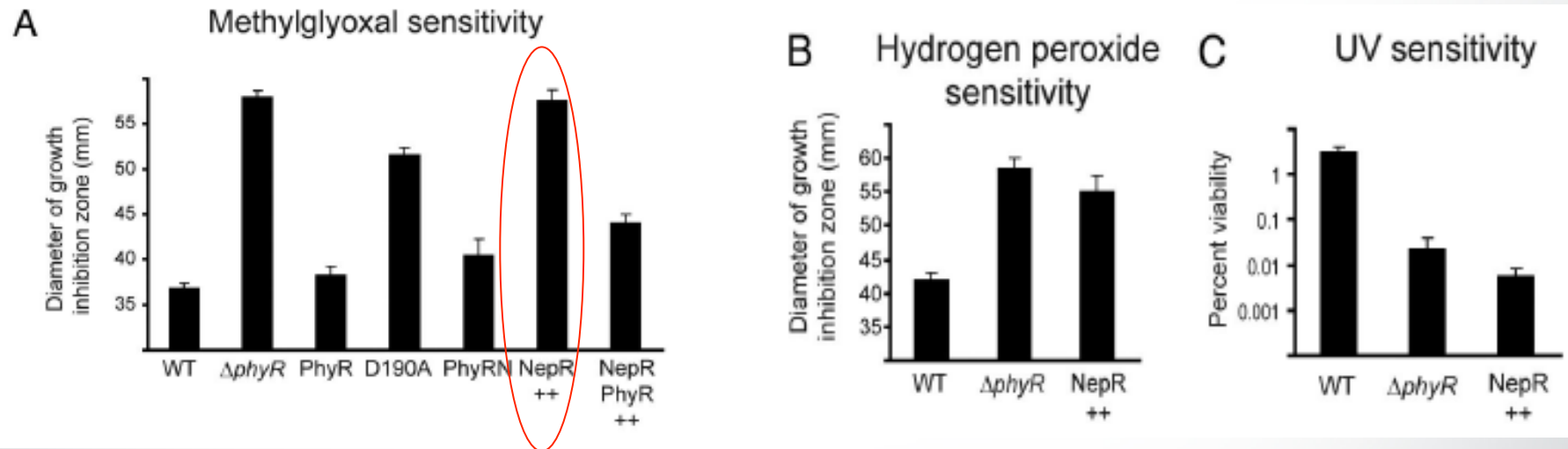
PhyR-Dependent Response is Negatively Regulated by NepR

- No DNA-binding activity or an interaction with RNA polymerase of phosphorylated or unphosphorylated PhyR or its ECF sigma factor domain was shown.

NepR- a candidate protein involved in regulatory cascade:

- It is conserved and found in synteny with *phyR* in Alphaproteobacteria
- The NepR homologue SMc01505 is a negative regulator of the ECF sigma factor RpoE2 in *S. meliloti*.

PhyR-Dependent Response is Negatively Regulated by NepR-Overexpression



PhyR-Dependent Response is Negatively Regulated by NepR

Among 263 genes differentially regulated when NepR was overproduced, 114 were also part of the PhyR regulon

Table 1. Genes of PhyR, NepR, and σ^{EcfG1} regulons*

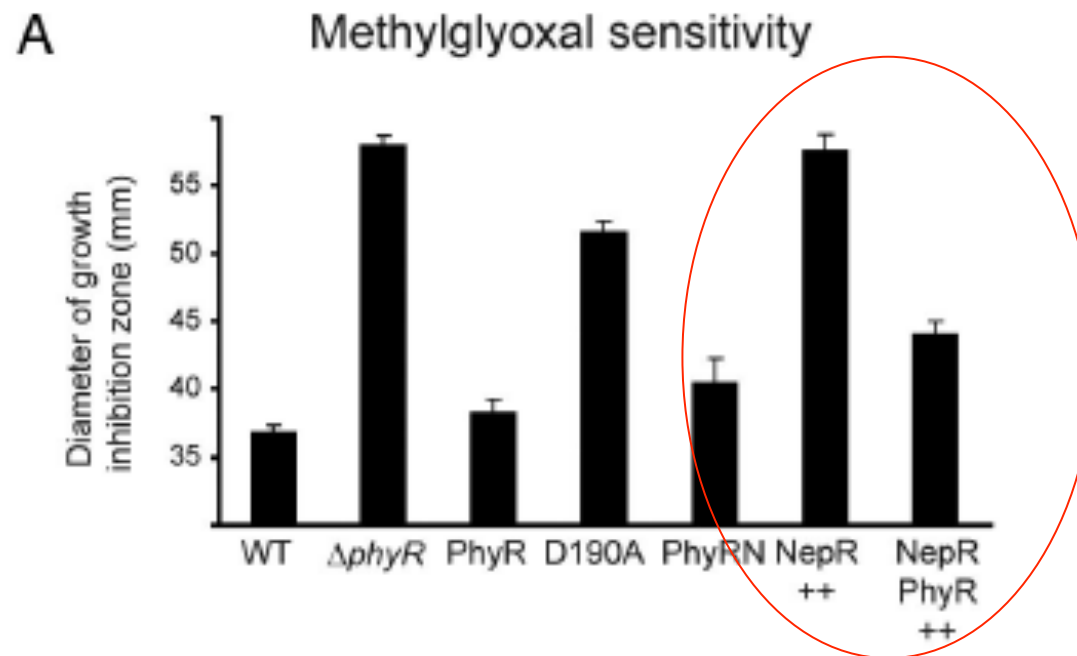
Function (gene homologue)	Gene	Fold change <i>phyR</i> OE/ Δ <i>phyR</i> [†]	Fold change <i>nepR</i> OE/WT [†]	Fold change Δ <i>ecfG1</i> /WT [†]
Detoxification/protection against stresses				
Catalase (<i>katE</i>)	RMQ09549	10.2	-7.4	-7.9
Lactoylglutathione lyase (<i>gloA</i>)	RMQ01575	4.9	-3.7	-2.1
Lactoylglutathione lyase (<i>gloA</i>)	RMQ02894	6.1	-4.4	-2.7
Glutathione S-transferase (<i>yqjG</i>)	RMQ06706	4.7	-2.2	-3.2
Glycogen debranching enzyme (<i>glgX</i>)	RMQ05904	7.0	-4.2	-4.4
DNA protection during starvation protein (<i>dps</i>)	RMQ05258	12.1	-7.7	-5.7
Regulation				
RNA polymerase σ 32 factor (<i>rpoH</i>)	RMQ12010	9.0	-4.3	-8.0
Transcriptional regulator CRP/FNR family	RMQ08139	24.6	-6.4	-10.2

OE, overexpression; WT, wild type.

*Twenty-six additional genes are not presented in this table. For a complete list, see [Table S1](#).

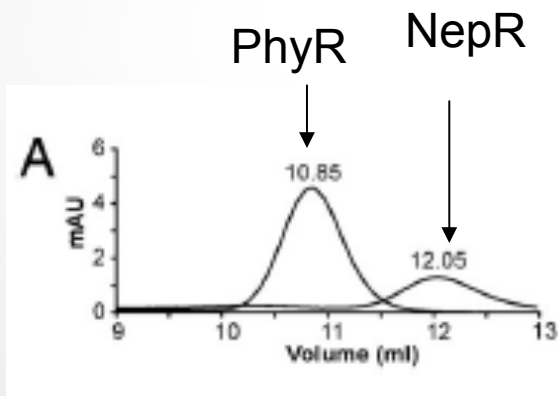
[†]Average changes of 3 experiments.

Suppressing Effects of PhyR and NepR



Overexpression of *phyR* suppresses the effect of NepR

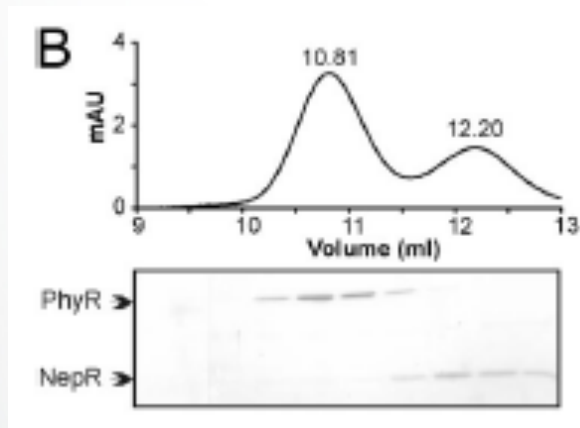
Phosphorylated PhyR Interacts with NepR via its ECF Sigma Factor-like Domain



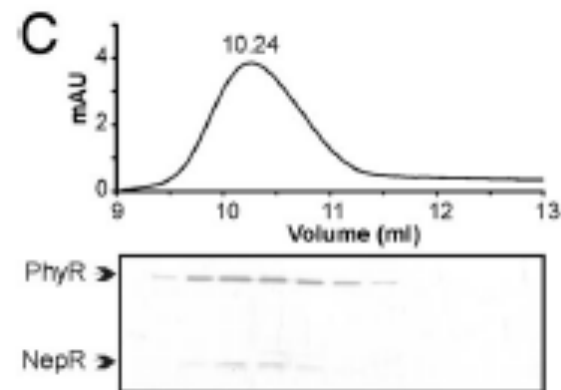
Elution profile of PhyR and NepR passed separately over Superdex 75 10/300

- Apparent molecular mass of PhyR is ~32 kDa
- PhyR remains in its monomeric form when phosphorylated
- NepR is found as homodimer (predicted MW 11.8 kDa)

Phosphorylated PhyR Interacts with NepR via its ECF Sigma Factor-like Domain

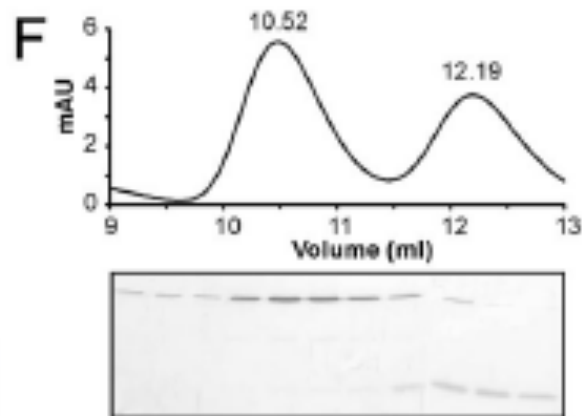


Nonphosphorylated PhyR
incubated with NepR before
loading



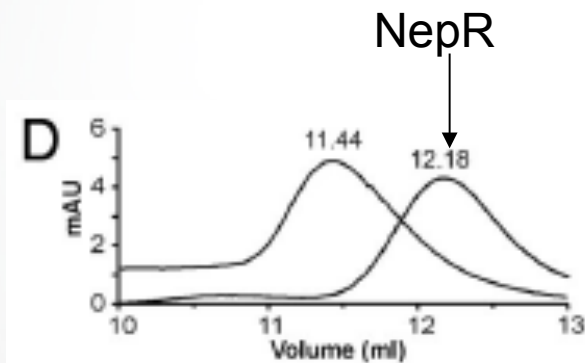
PhyR phosphorylated
with acetyl phosphate
incubated with NepR
before loading

Phosphorylated PhyR Interacts with NepR via its ECF Sigma Factor-like Domain

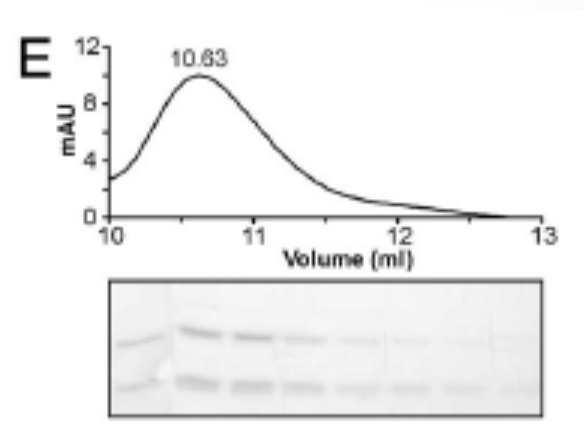


Mutant PhyRD190A incubated with NepR with or without acetyl phosphate

NepR binds to ECF Sigma factor domain of PhyR

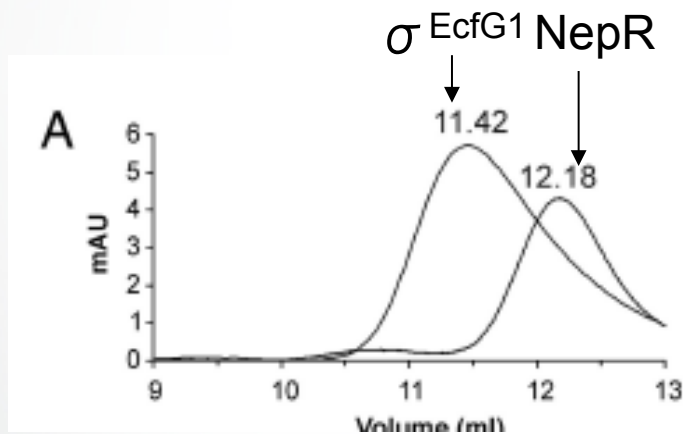


ECF Sigma Factor domain of PhyR and NepR passed separately

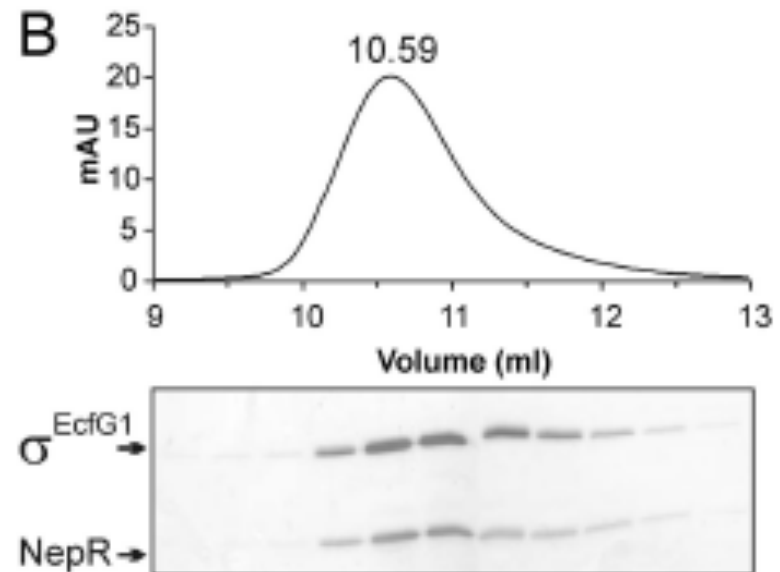


ECF Sigma Factor domain of PhyR and NepR passed after incubation

NepR is an Anti-Sigma Factor of σ^{EcfG1}

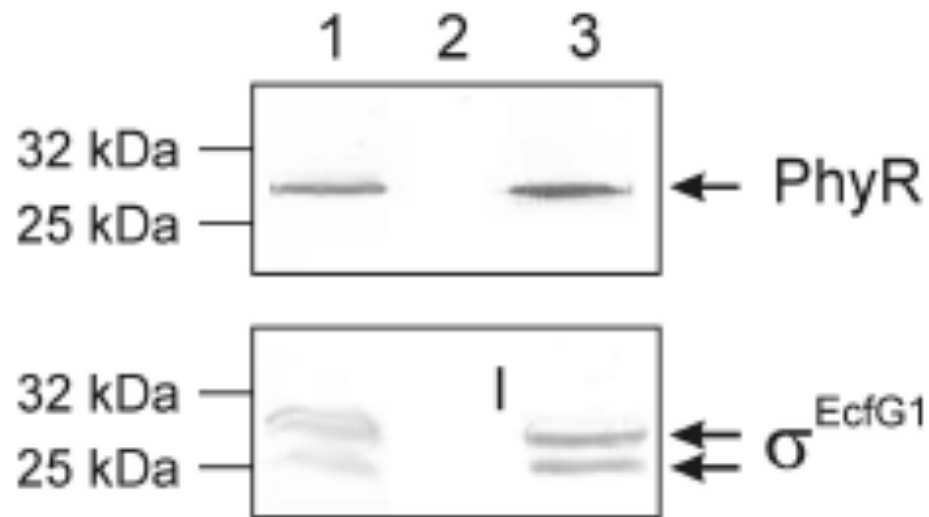


Truncated σ^{EcfG1} and NepR passed separately



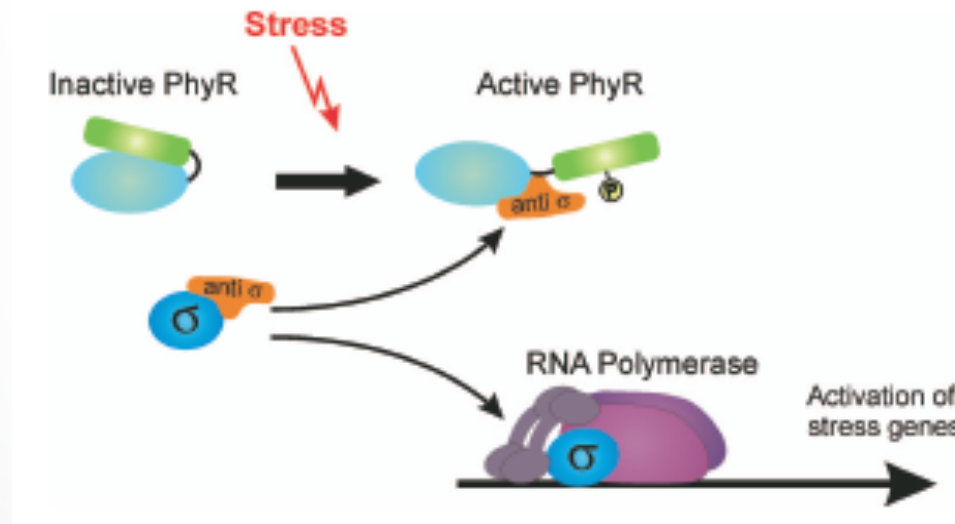
Truncated σ^{EcfG1} and NepR passed after incubation separately

NepR interacts in vivo with PhyR and σ^{EcfG1}



Recombinant NepR protein was overproduced in *M. extorquens* AM1 and purified by Ni-NTA via C-terminal 6X his-tag. Crude extract (lane 1, washing step (lane 2) and elution fraction were analyzed by Western and detected by polyclonal antibodies of anti-PhyR and anti- σ^{EcfG1}

Model for Partner Switching



Questions??

Thanks...