



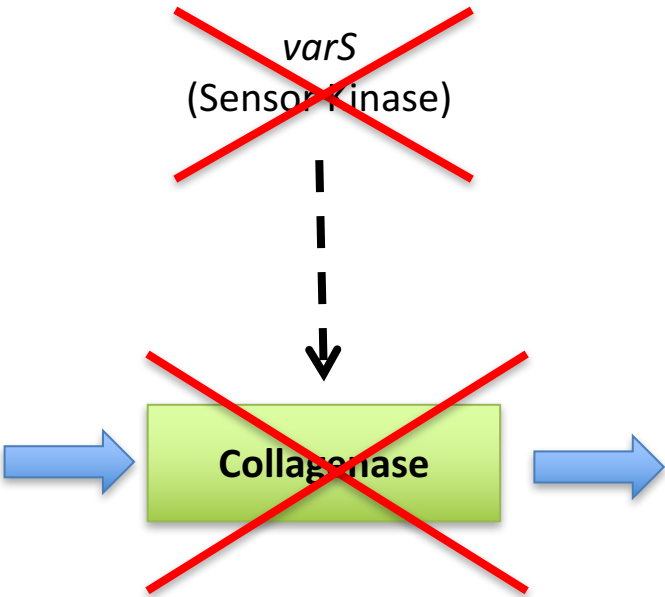
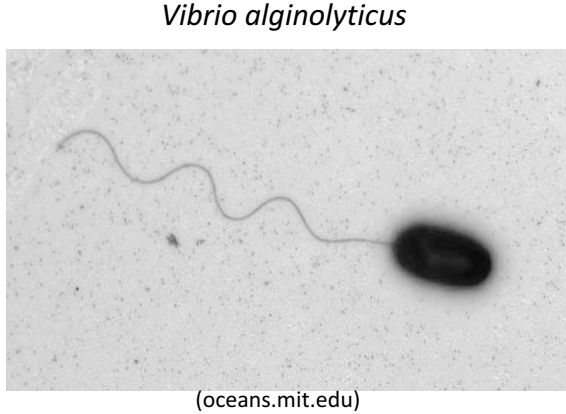
Regulation of small regulatory RNA CsrB in *Vibrio alginolyticus* and its role in collagenase production

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Introduction



Necrotizing Fasciitis Due to *Vibrio alginolyticus* in an Immunocompetent Patient



Juan M. Gomez et al. J. Clin. Microbiol. 2003;41:3427-3429

- Gram negative bacteria.
- Marine bacteria.

Purposes of Study

VarS
(Sensor Kinase)

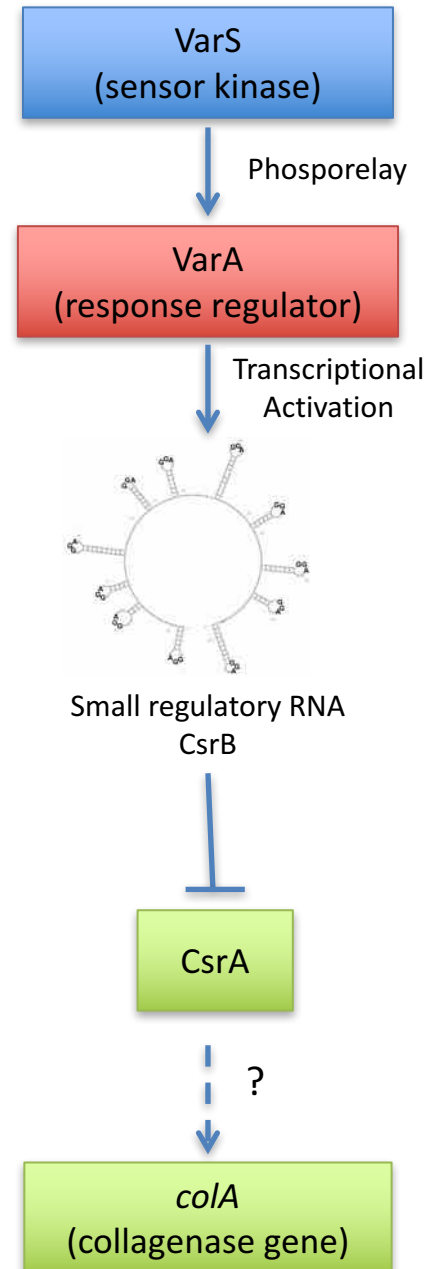


Collagenase

Purposes of Study

- To identify CsrB in *V. alginolyticus*.
- To Examine whether VarS/VarA two-component system regulates the expression of *csrB* in *V. alginolyticus*.
- To determine the role of CsrB in collagenase production.

We hypothesized that VarS/VarA two-component system regulates collagenase production through small regulatory RNA CsrB in *V. alginolyticus*



Identification of CsrB

VarA-binding site

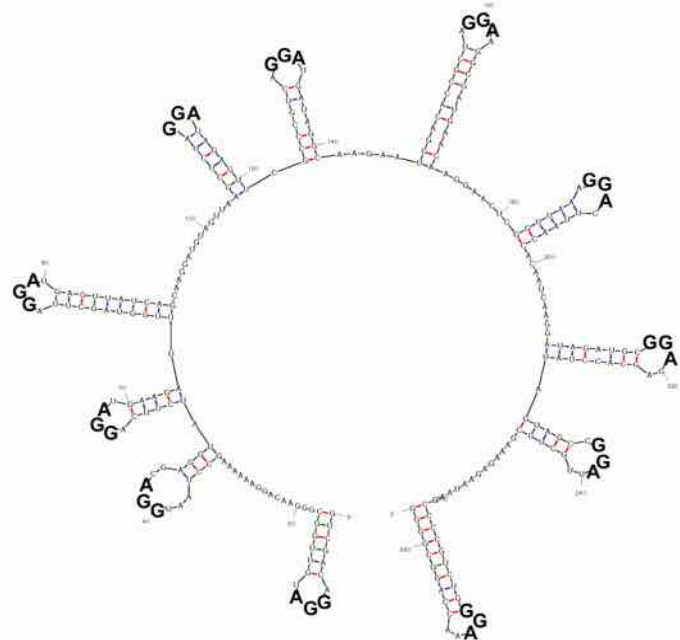
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TGTGCGACATCTCTTACAAGTAATGTAAGTAAAACGA  
CCATCATTGACTGAGACAAAATCGAAAAATTTAATATA  
AAGCATTGAAAATTATAACTTATACTATTAAACAA  
TATTCGCTGCCAAAGTTTTTTTACCTAGCTAATTGAG  
AGTAATCAGCAAACATCAATACTGATTGTGTCGACA  
GGA TGTGGCGGGAACAGGA AAAAAGCCTAATGGACG  
AGGTATCTTCAGGATGAAGATTTGGTAGCTTAGGATG  
AGTTATCAGCAAGGATGTAGTTAAGCTCTAGGATAGA  
GTTCCGCCGTCAAGGATGATAGGCAAGAATGGACACCG  
CTAGGAGGCGATGAACAAGGAACTCGGTTAAAGGAC  
TTAACCACAATCAAGGATAGATGCAGGGAGCACCTAT  
AGTAGCCGGATTGCTGCGAAAGAGAATAAGCCCCGTC  
TGGAAACCAGGCGGGG
```

csrB in *V. alginolyticus* I.029 :

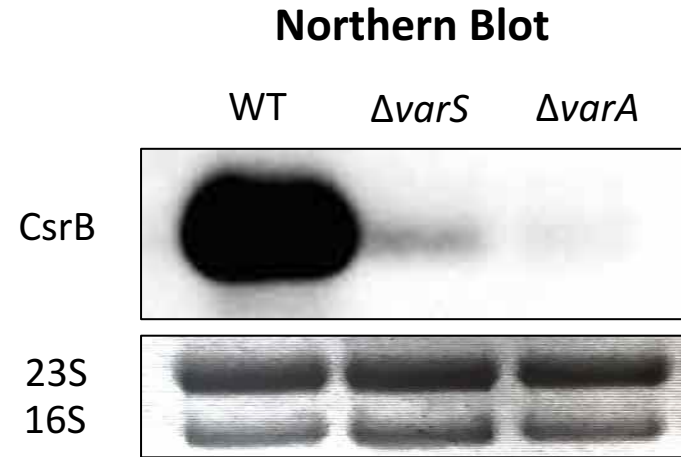
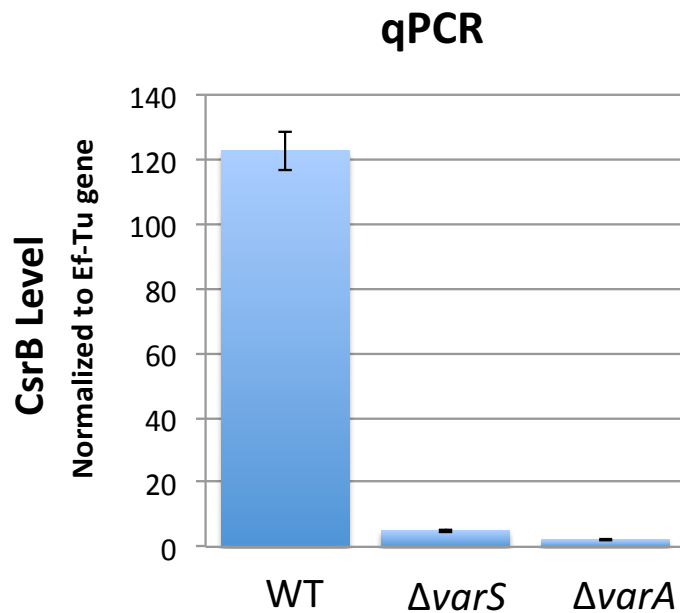
- Contain high frequency of GGA sequence.
- Transcription start site (TSS) of *csrB* was determined using 5'-rapid amplification cDNA end (RACE) method.
- The consensus sequence for VarA-binding was found in promoter region.

Secondary structure of the CsrB:

- Predicted using the RNA folding software Mfold.
- Consist of 11 hairpin structures.
- Most of GGA motifs are located in loop structure.

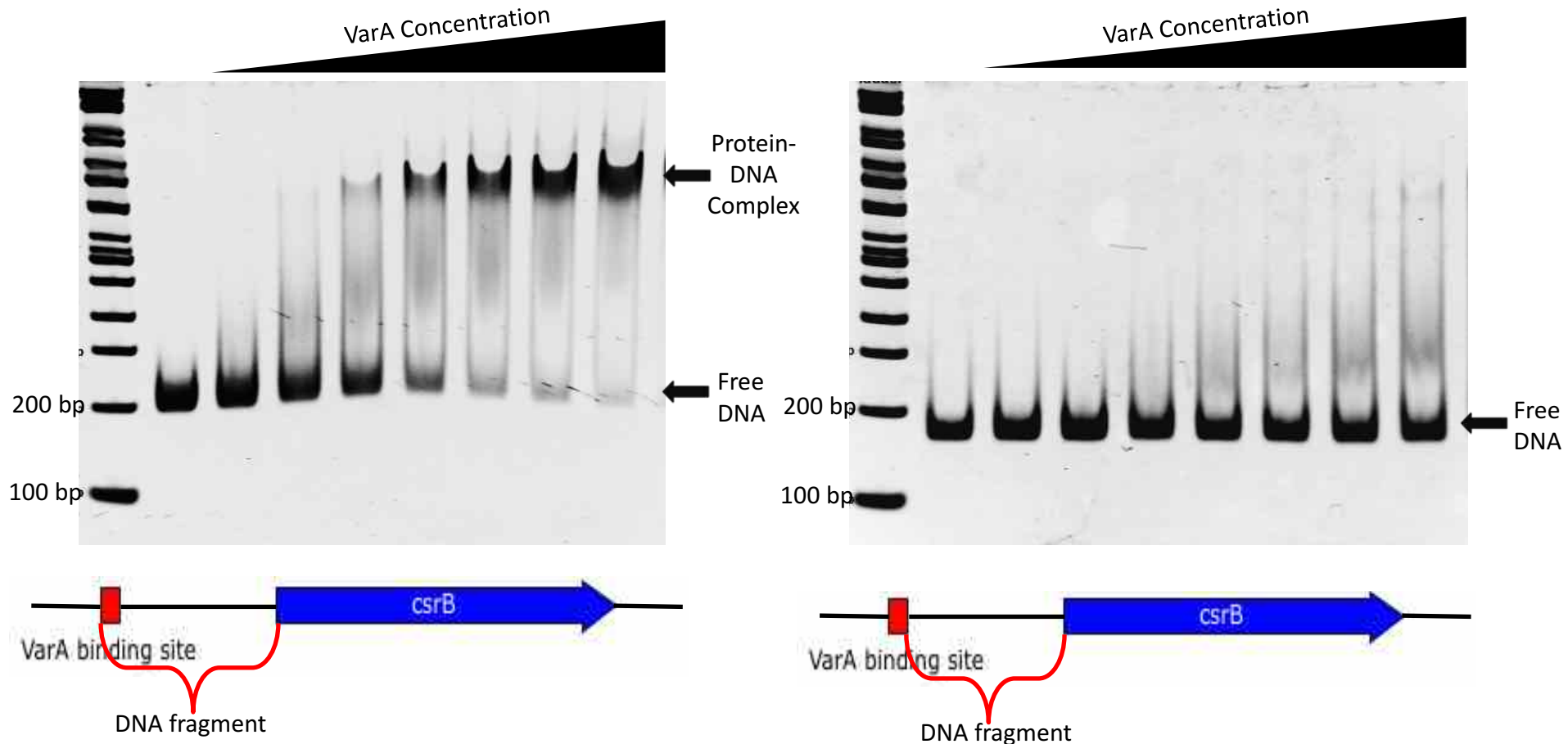


VarS/VarA positively regulates *csrB* expression



Deletion of either *varS* or *varA* significantly reduced the expression of *csrB*, indicating that the transcription of *csrB* is positively controlled by VarS/VarA two-component system.

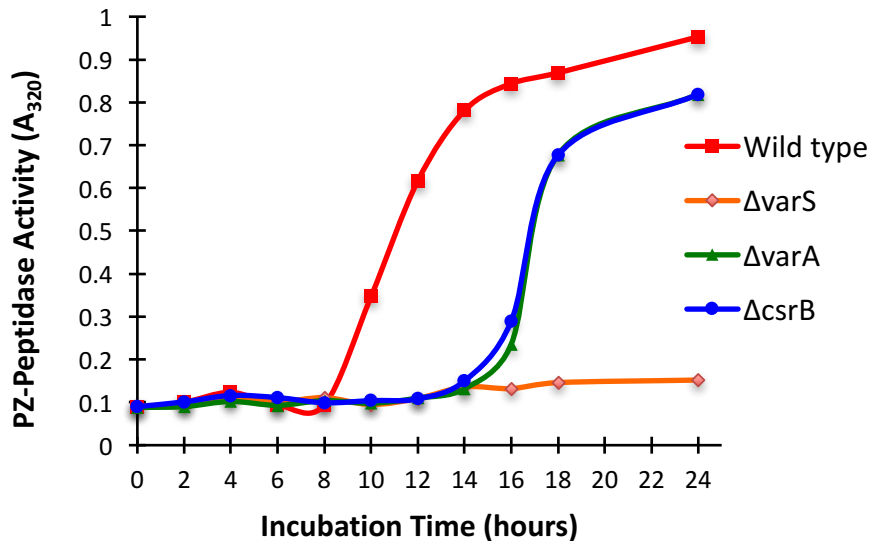
VarA binds to promoter region of *CsrB*



Electrophoretic Mobility Shift Assay (EMSA) experiment showed the binding of VarA to *csrB* promoter region, suggesting that VarA directly regulates *csrB* expression

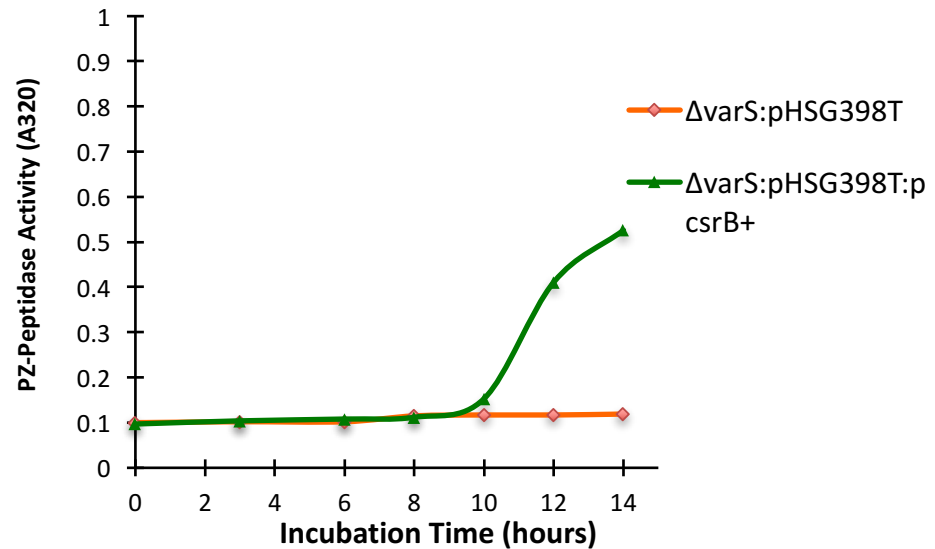
CsrB regulates the production of collagenase

Collagenase production
in Wild type, $\Delta varS$, $\Delta VarA$ and $\Delta CsrB$



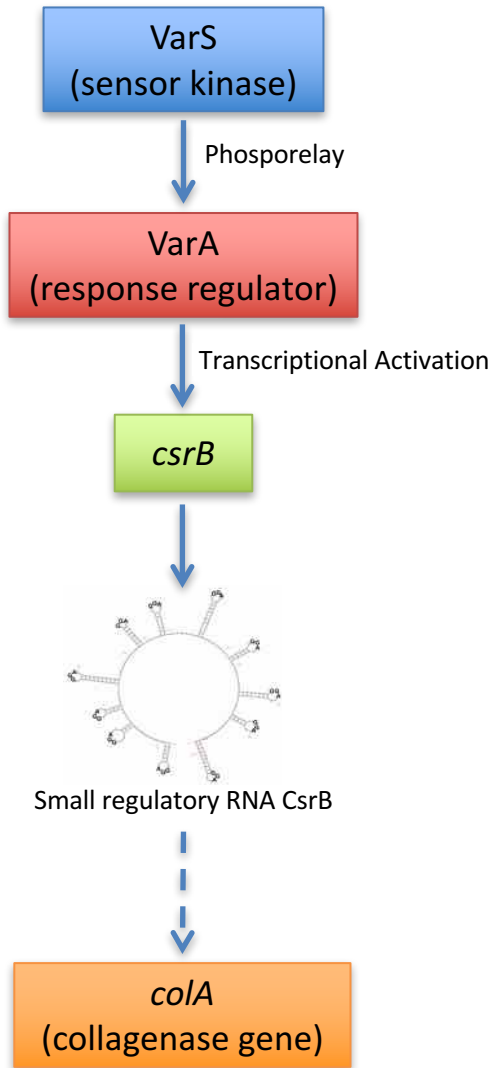
Consistent with the deletion of *varA*, deletion of *csrB* partially suppressed the collagenase production.

Collagenase production
in Overexpression of *csrB* in $\Delta varS$ strain



Introduction of *csrB* expression vector restored the deficiency in collagenase production in *varS* deletion mutant.

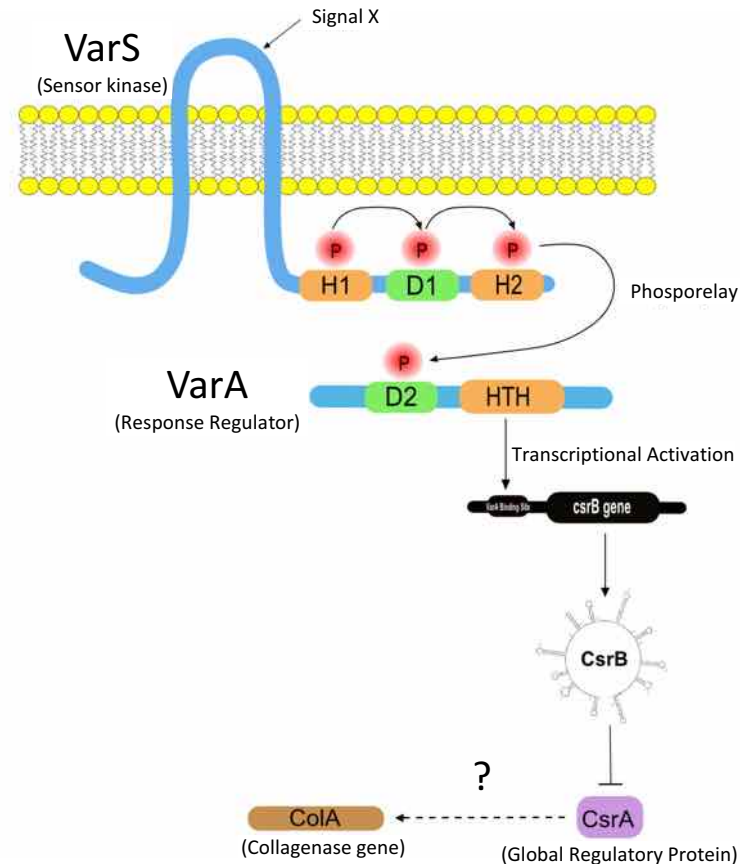
Conclusions



- VarS/VarA two component system positively regulates *csrB* expression.
- VarA directly regulates the expression of small regulatory RNA CsrB.
- CsrB regulates the production of collagenase in *V. alginolyticus*.

Purposes of Study

- To identify CsrB in *V. alginolyticus*.
- To Examine whether VarS/VarA two-component system regulates the expression of *csrB* in *V. alginolyticus*.
- To determine the role of CsrB in collagenase production.



We hypothesized that VarS/VarA two-component system regulates collagenase production through small regulatory RNA CsrB in *V. alginolyticus*