

MopA and MopB and MOP

Specific Interactions between Four Molybdenum-Binding Proteins Contribute to Mo-Dependent Gene Regulation in *Rhodobacter capsulatus*

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Rhodobacter capsulatus

- Alpha-protobacterium
- Phototrophic Purple Bacterium
- Can grow aerobically and anaerobically
- Respiration and Photosynthesis
- Contains Mo-Enzymes
 - FeMoco Nitrogenase
 - Moco DMSO Reductase

facebook

[Sign Up](#) Sign up for Facebook to join Rhodobacter capsulatus fan club.

Rhodobacter capsulatus fan club

Global

Basic Info

Name: Rhodobacter capsulatus fan club
Category: Common Interest - Beauty
Description: A much-needed fan club for the awesomest alpha-proteobacteria ever!!-

Members

Displaying 8 of 29 members



Imene



Rebecca



Mustafa



Juliet



Abdulmecit



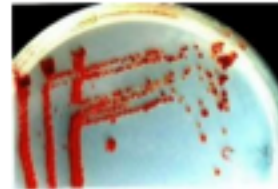
Brandon



Annette



Brian



The Wall

Displaying 5 of 15 wall posts.

[See All](#)



Alexander wrote at 9:45am on October 9th, 2009

RCV medium (Beatty 1981) isn't really selective for Rhodobacter, but as most other lab strains (like E.coli) grow quite slowly (if at all) on it it generally serves the purpose. You could increase selectivity even further by growing them anaerobically/phototrophically.

What do you need the selectivity for? I might be of more use to you if I had more details.



Anupama wrote at 10:50pm on August 30th, 2009

Hello!
Can anyone give me a suggestion that which medium is selective for Rhodobacter. Help would be appreciated!
Thanks



Brandon wrote at 8:40am on August 14th, 2009

I can't believe people DON'T join this.



Ryan wrote at 5:32pm on June 8th, 2008

I can't believe people join this.....

Group Type

Open: All content is public.

Officers

Krista
Rhodobacter's number one fan
Amanda
Rhodobacter lover
Mike
Rhodobacter enthusiast

Admins

• Mike (creator)

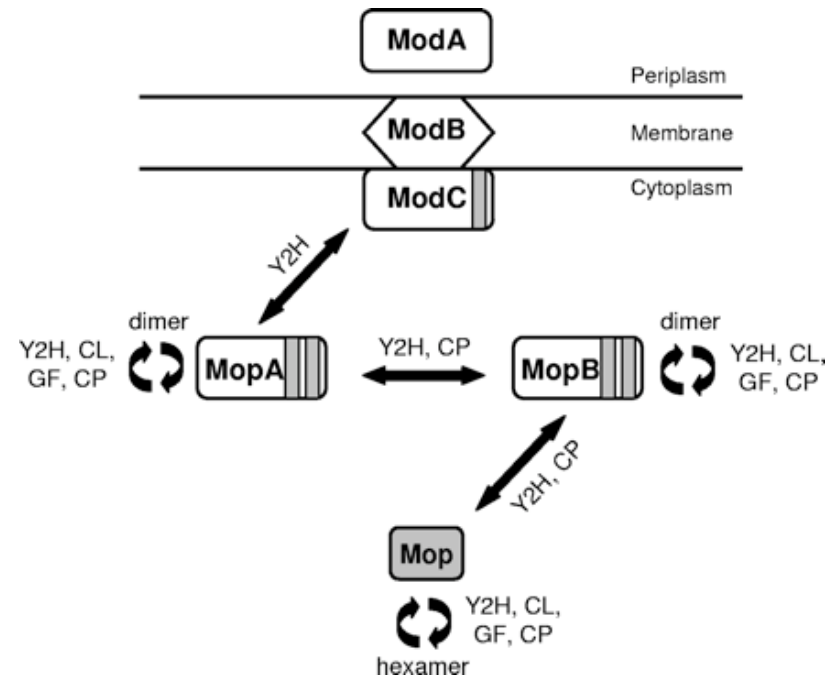
Regulation of Mo Transport

ModABC: Transports Molybdenum

MopA: Represses *mopAmodABCD* +Mo
 Activates *mop* +Mo

MopB: Represses *mopAmodABCD* +Mo
 Does not regulate *mop*
 Not regulated by Mo

Mop: Mo storage protein



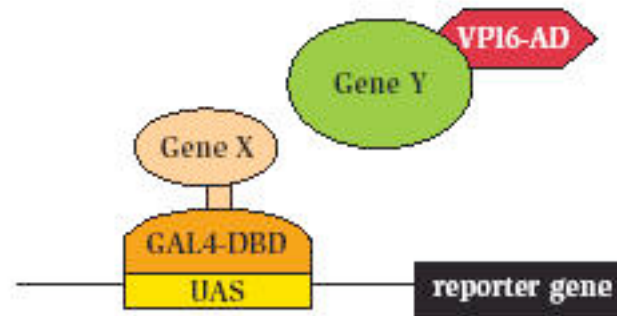
R. capsulatus contains 2-ModE like Regulators

Do these proteins interact?

If so, do they influence Mo-dependent regulation?

Yeast-Two-Hybrid

A.



B.

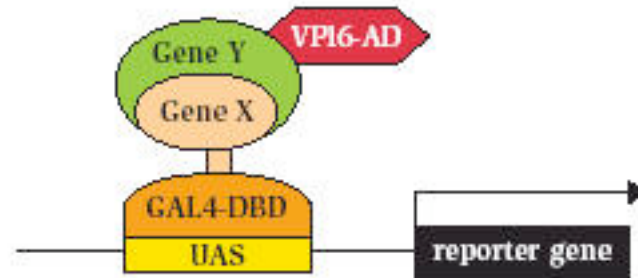


Figure 2: Yeast-Two-Hybrid System

MopA Interacts with:
MopA
MopB

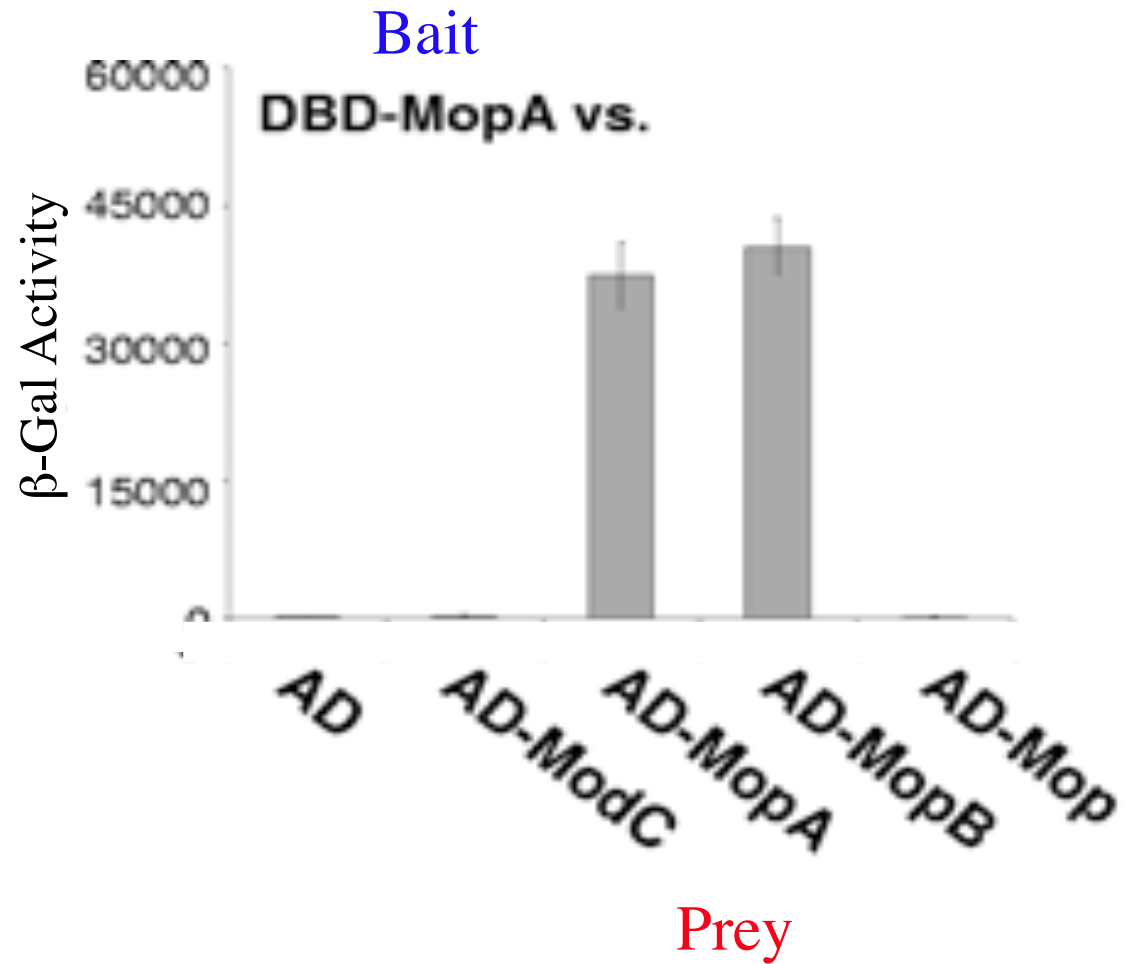


Figure 2: Yeast-Two-Hybrid System

MopB Interacts with:
MopA
MopB
Mop

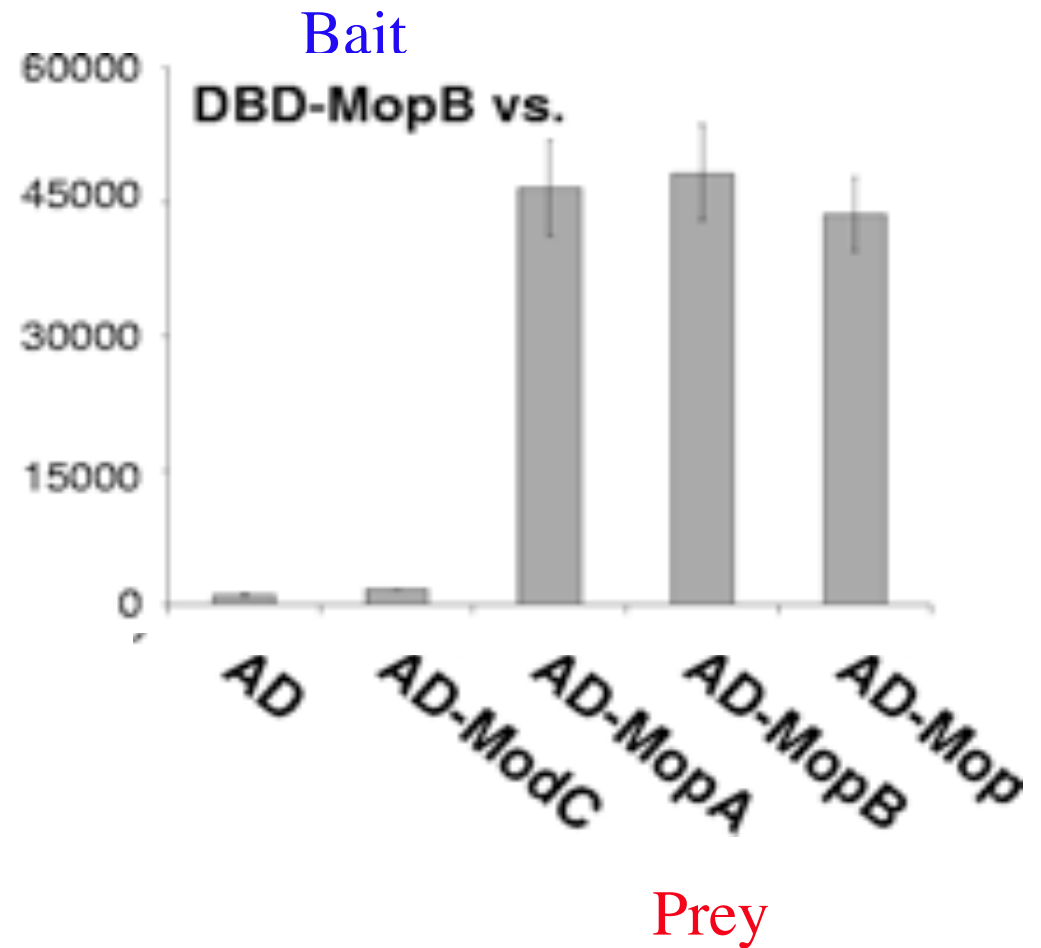


Figure 2: Yeast-Two-Hybrid System

Mop Interacts with:
MopB
Mop

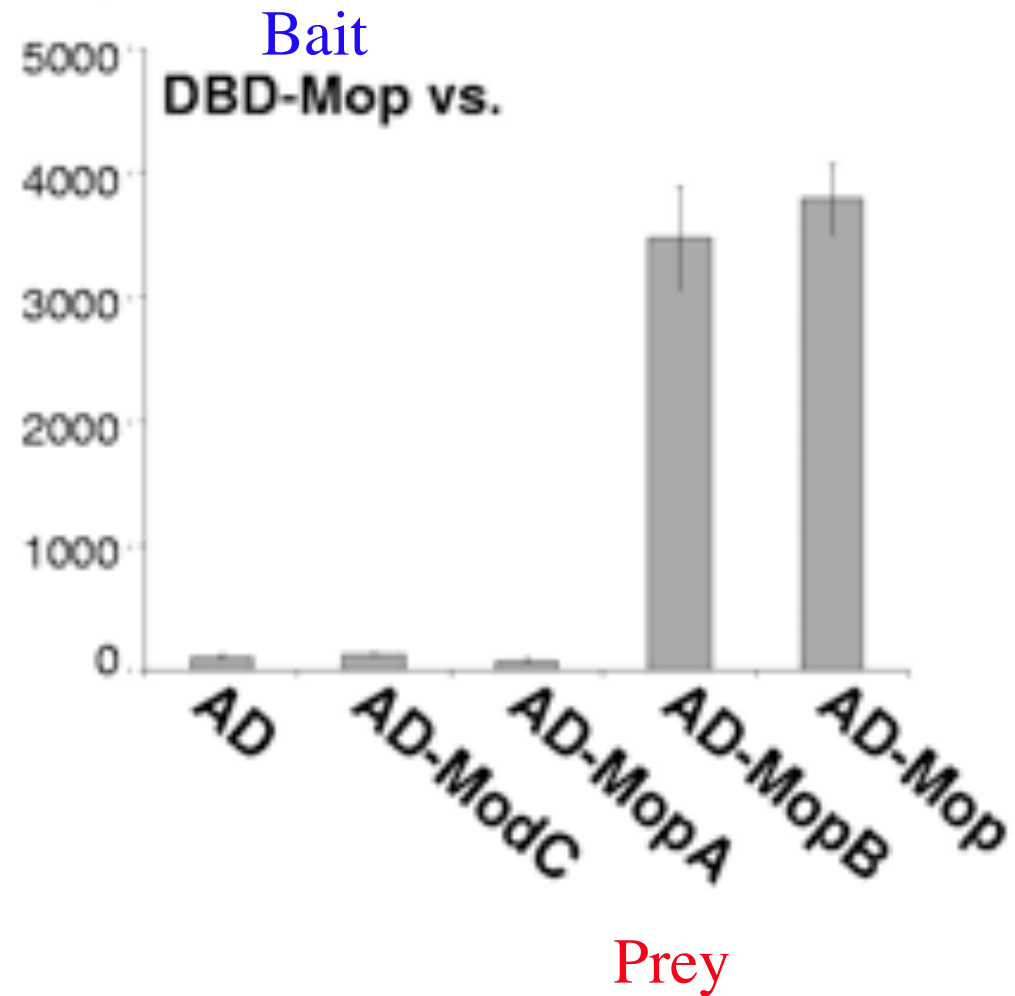
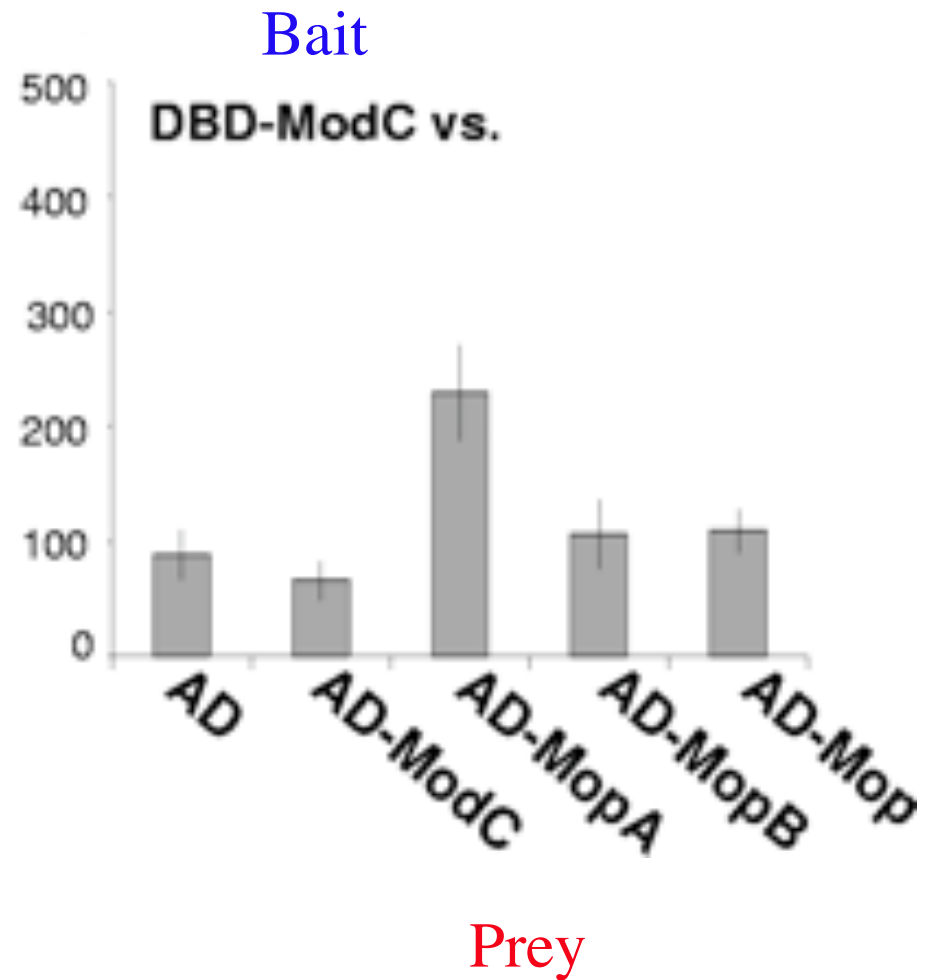


Figure 2: Yeast-Two-Hybrid System

ModC Interacts with:

MopA

*Only when ModC
is used as Bait



Co-Purification

- His-Tagged and Strep-Tagged the Mop Proteins
- Co-transformed combinations of Mop Proteins and Overexpressed them in *E. coli*
- Ran Crude extracts on a Ni-NTA column
 - Eluted bound proteins with Imidazole
 - Identified proteins using Anti-His / Anti-Strep Ab

Theory: Only His-Proteins should stick to Ni-NTA
 If Strep-Protein is detected it must interact with co-
 expressed His-Protein in order to stick to Ni-NTA

Protein Purification Profiles

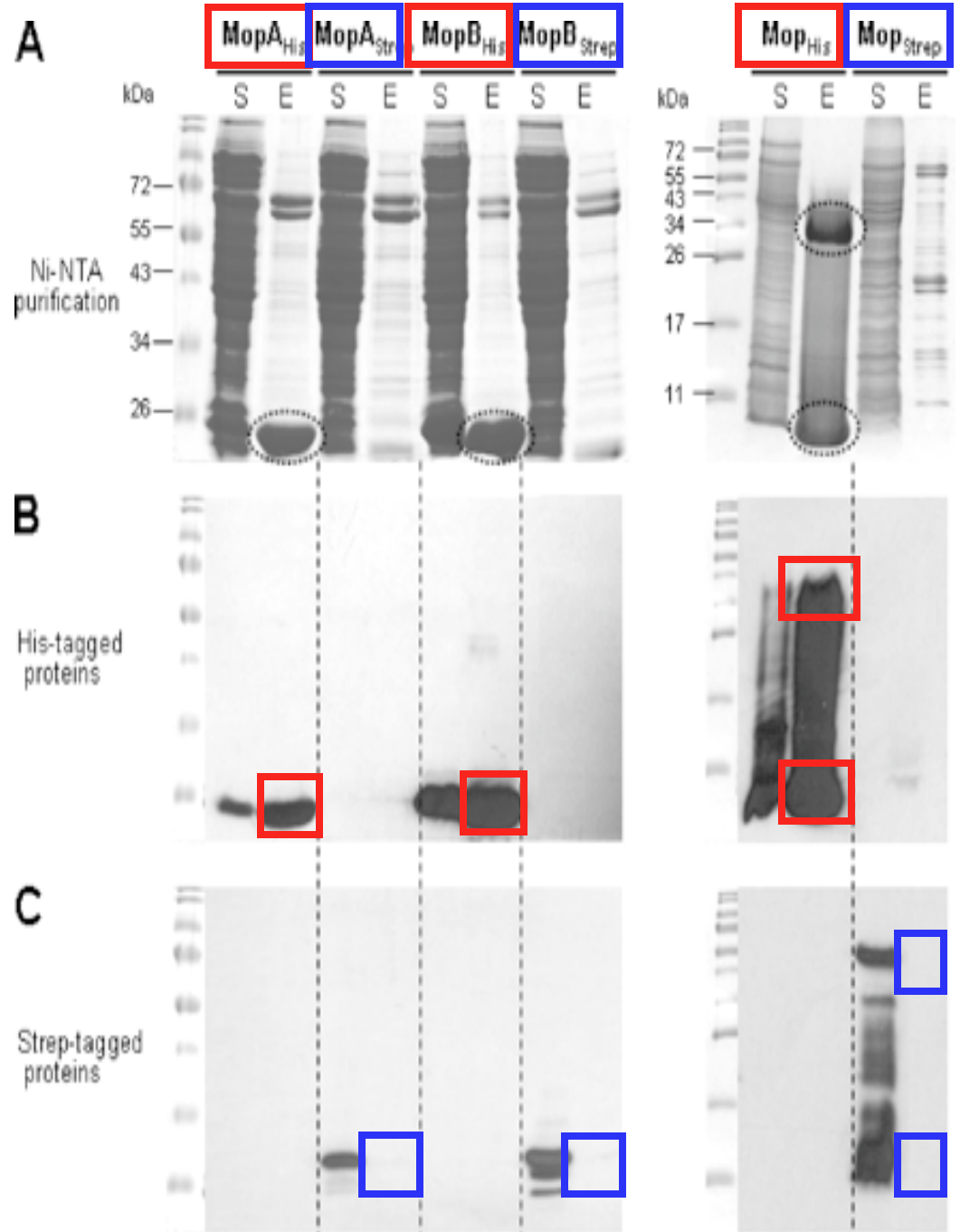
S- Crude extract

E- Eluates from Column

+ 1mM Mo

Anti-His Ab

Anti-Strep Ab



Homomer Formation

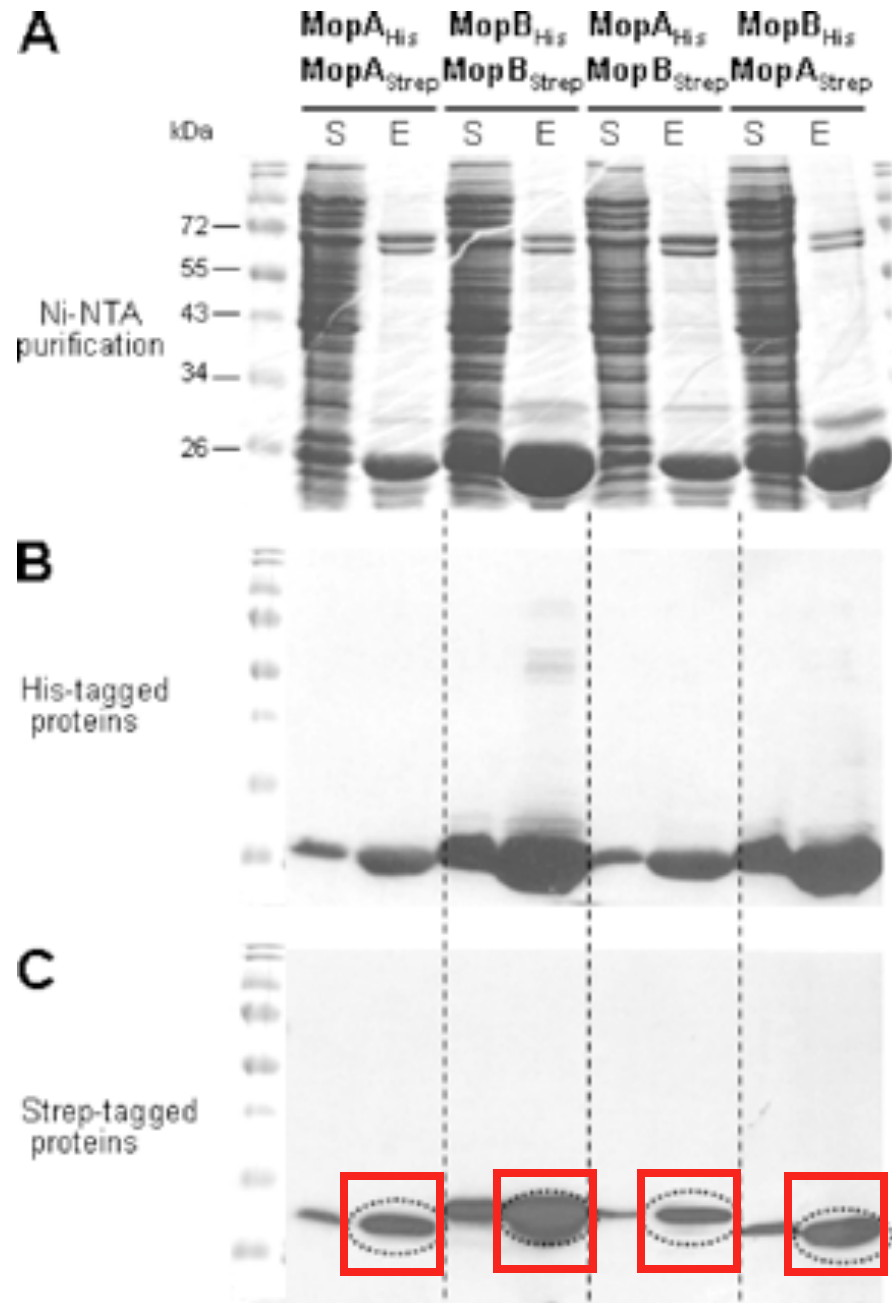
MopA - MopA

MopB - MopB

Heteromer Formation

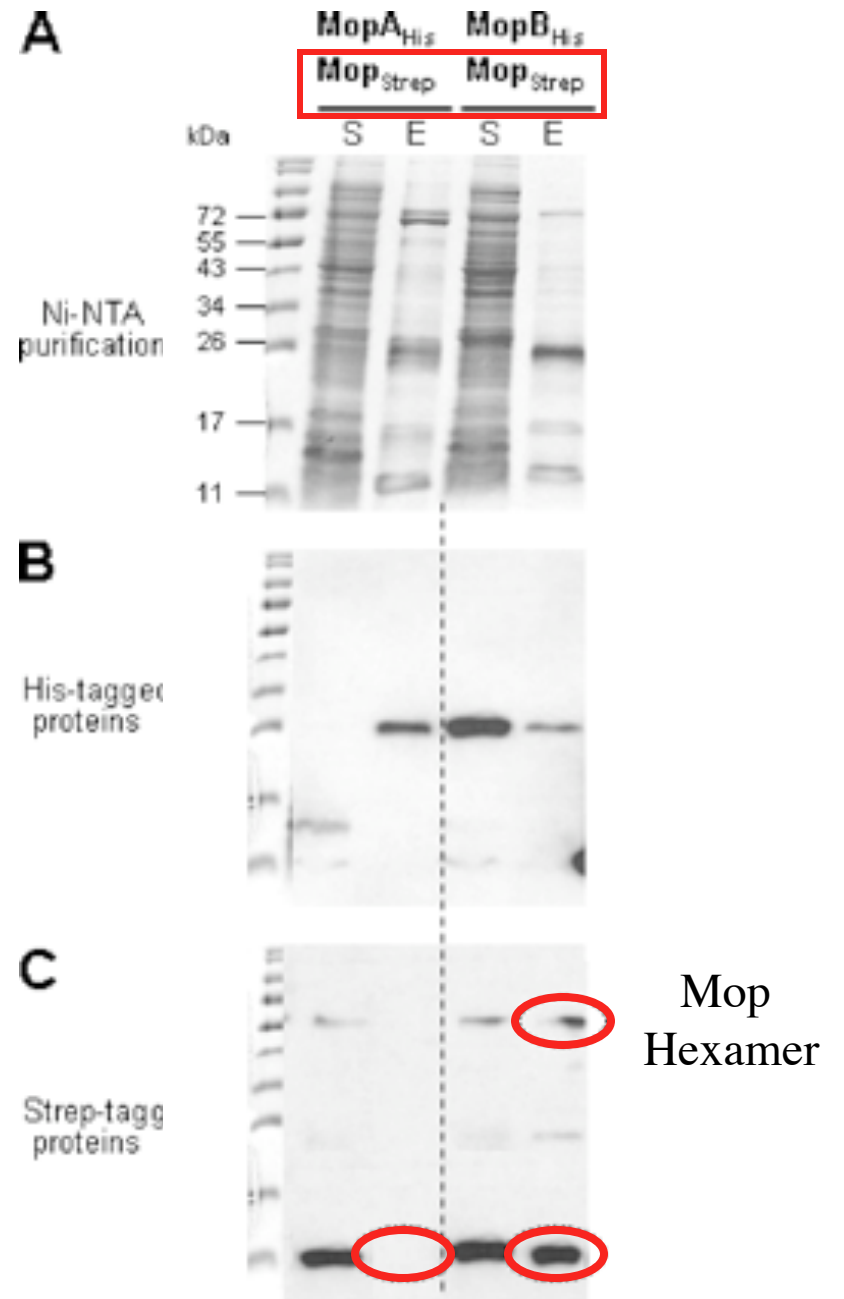
MopA - MopB

MopB - MopA



Mop Co-Purification

Mop - MopB ONLY



Protein-Protein Interactions

MopA

MopA

MopB

ModC

MopB

MopA

MopB

Mop

Mop

Mop

MopB

The *E. coli* ModE the Di-Mop domains (TOBE) are Primarily responsible for Dimerization (Through Crystal Structure)

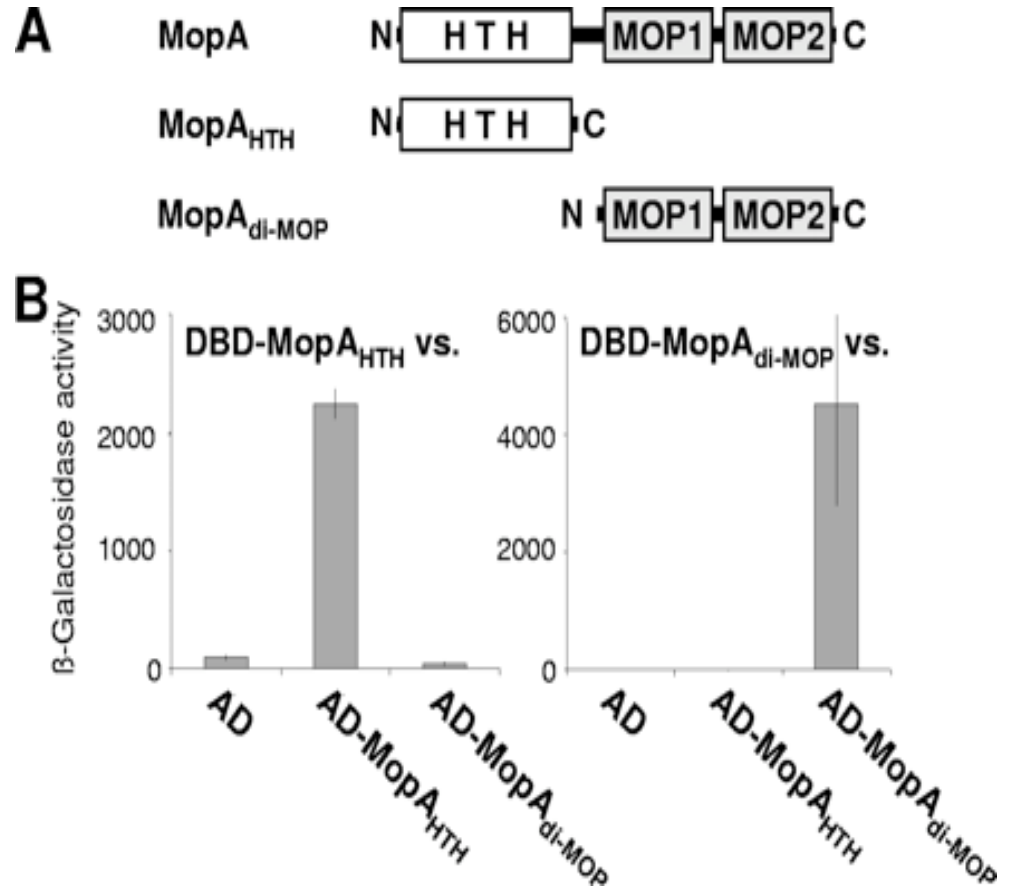
What domains are required in *R. capsulatus*?

Both Domains Contribute to Dimerization For MopA

Interactions:

MopA_{HTH} - MopA_{HTH}

MopA_{MOP} - MopA_{MOP}



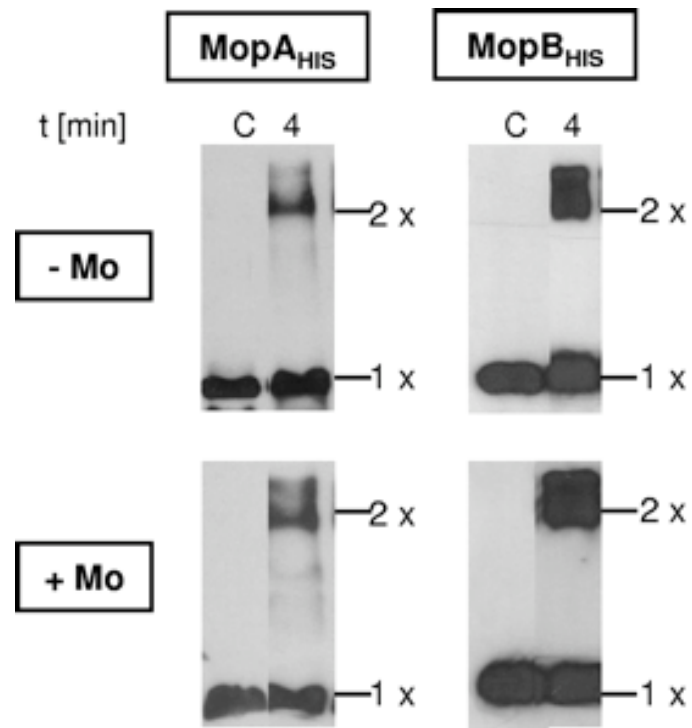
Why did they not look at MopB?

Mop Proteins Form Homomers

- What is the oligomeric state of these Mops?
- *E. coli* ModE is Dimeric
- *A. vinelandii* ModG is Trimeric (MOP)
- *C. pasteurianum* MopII is Hexamer (MOP)

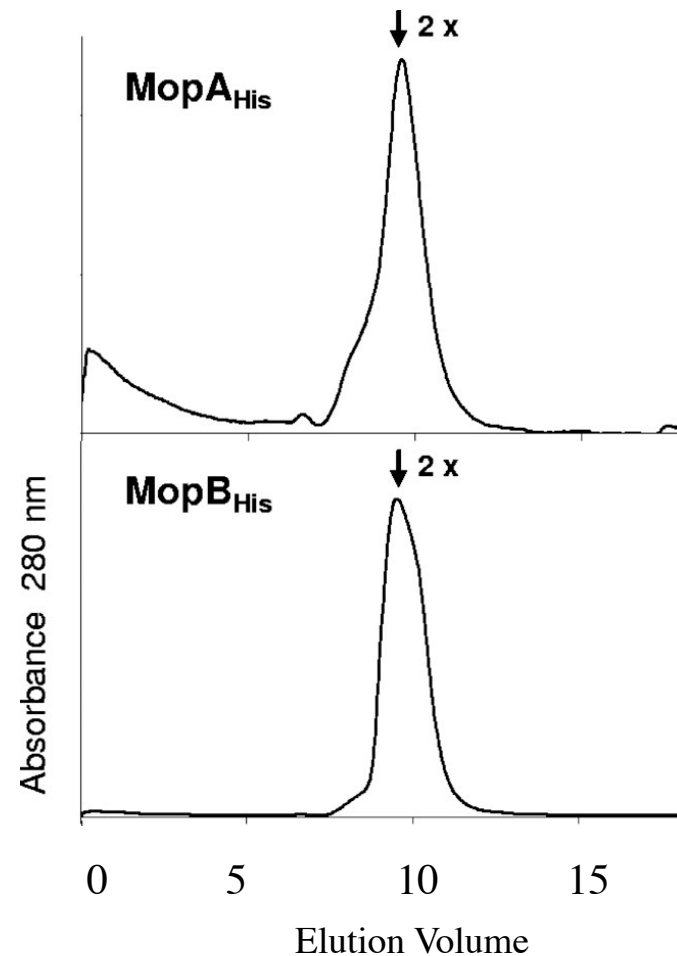
MopA and MopB Exist as Dimers Independent of Mo

Crosslinking Western Blots

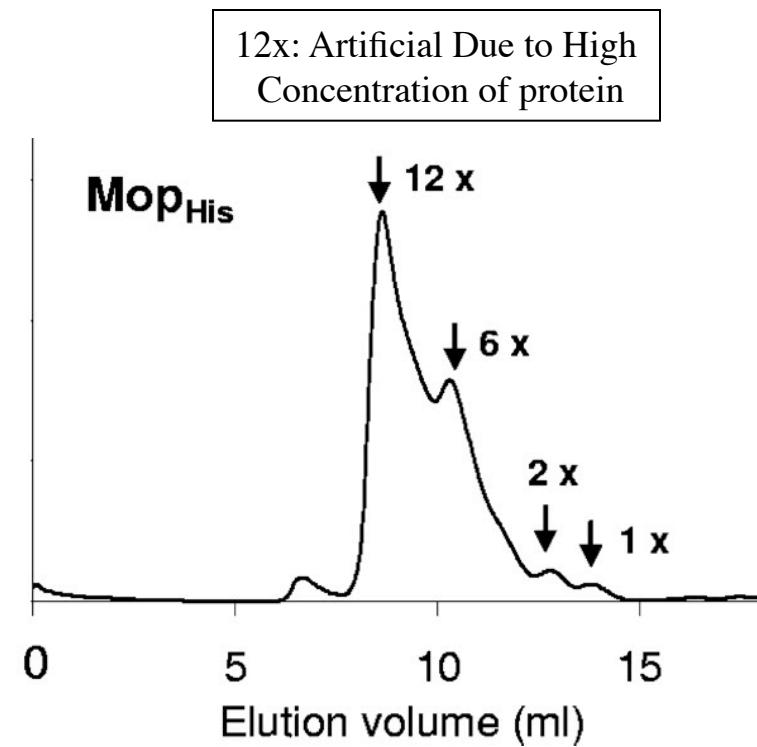
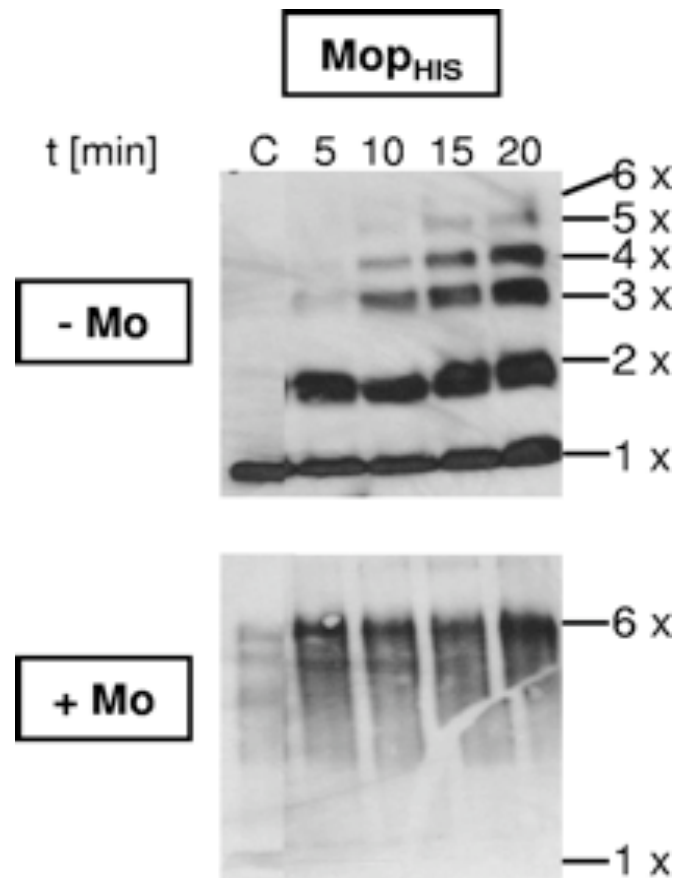


C = Control
No Crosslinking

Gel Filtration



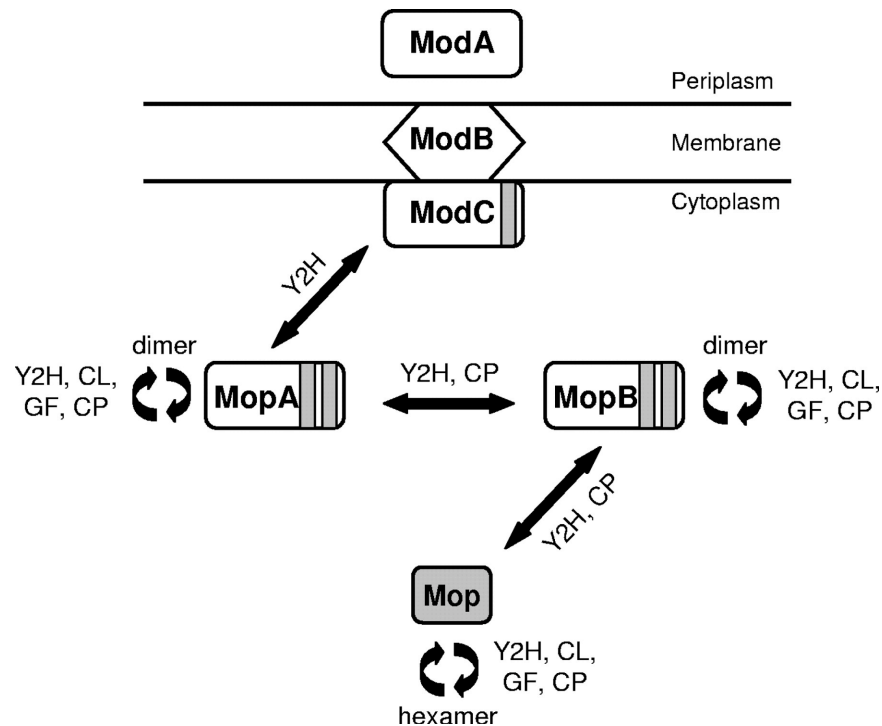
Mop Forms Complexes from Monomers to Hexamers



Timer of Dimers

TABLE 3. Mo-binding properties of *R. capsulatus* MOP Domain proteins

Protein	B_{\max} , mean \pm SD	K_D , mean \pm SD
MopA	1.83 ± 0.07	0.68 ± 0.06
MopB	1.69 ± 0.13	0.31 ± 0.09
Mop	1.10 ± 0.07	1.80 ± 0.50
ModC	1.08 ± 0.09	2.47 ± 0.73



- *mopA* Expression is Repressed by Mo
- *mopB* Unaffected by Mo
- MopA and MopB Ratio changes based on Mo availability

- Does MopA-MopB Dimer regulate Mo-Dependent genes?

- Looked at *mop*

Only Known Mo-gene that is Positively Regulated
Only activated when MopA binds promoter

- Does *mop* regulation change when:

Mo is present

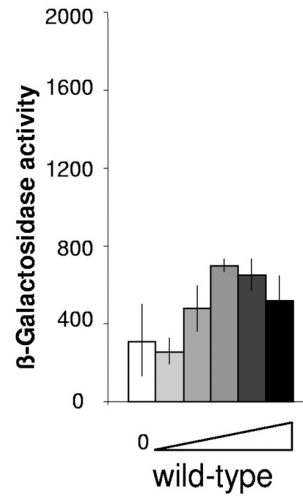
MopA is deleted

MopB is deleted

MopA and MopB are deleted

mop::lacZ

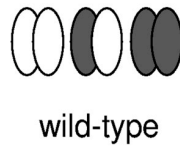
C



[Mo]

- 0
- 10nm
- 100nm
- 1 μ m
- 10 μ m
- 100 μ m

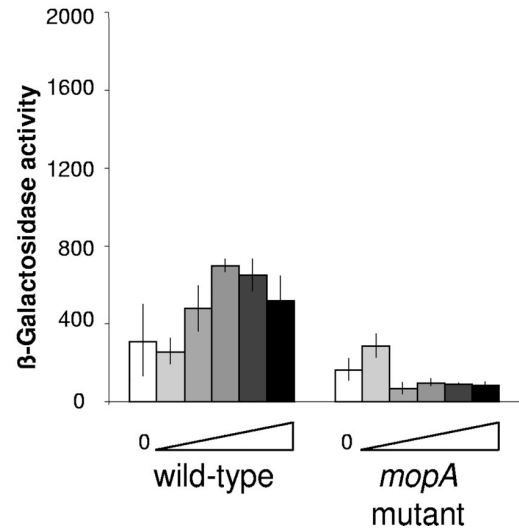
B



Mop is Activated when Mo is Present

mop::lacZ

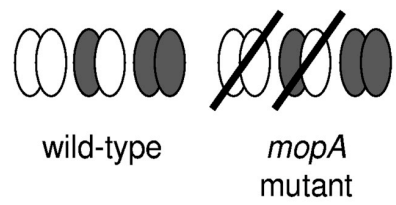
C



0
10nm
100nm
1 μ m
10 μ m
100 μ m

[Mo]

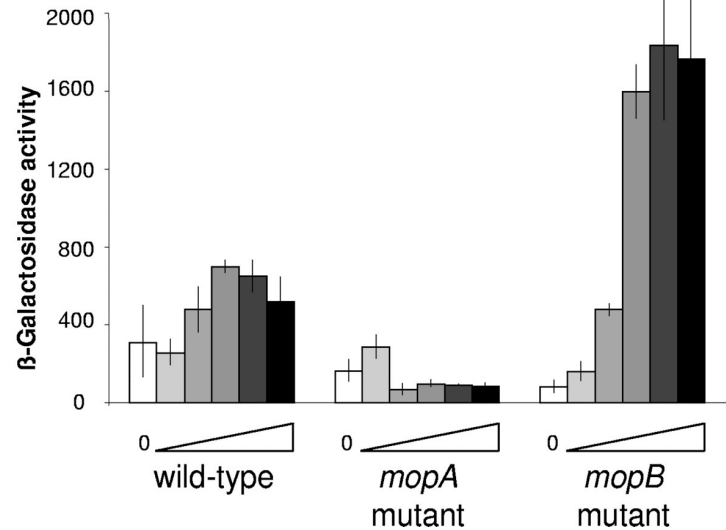
B



MopA Is Required for *mop* Transcription

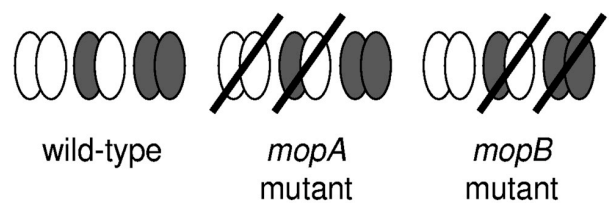
mop::lacZ

C



[Mo]

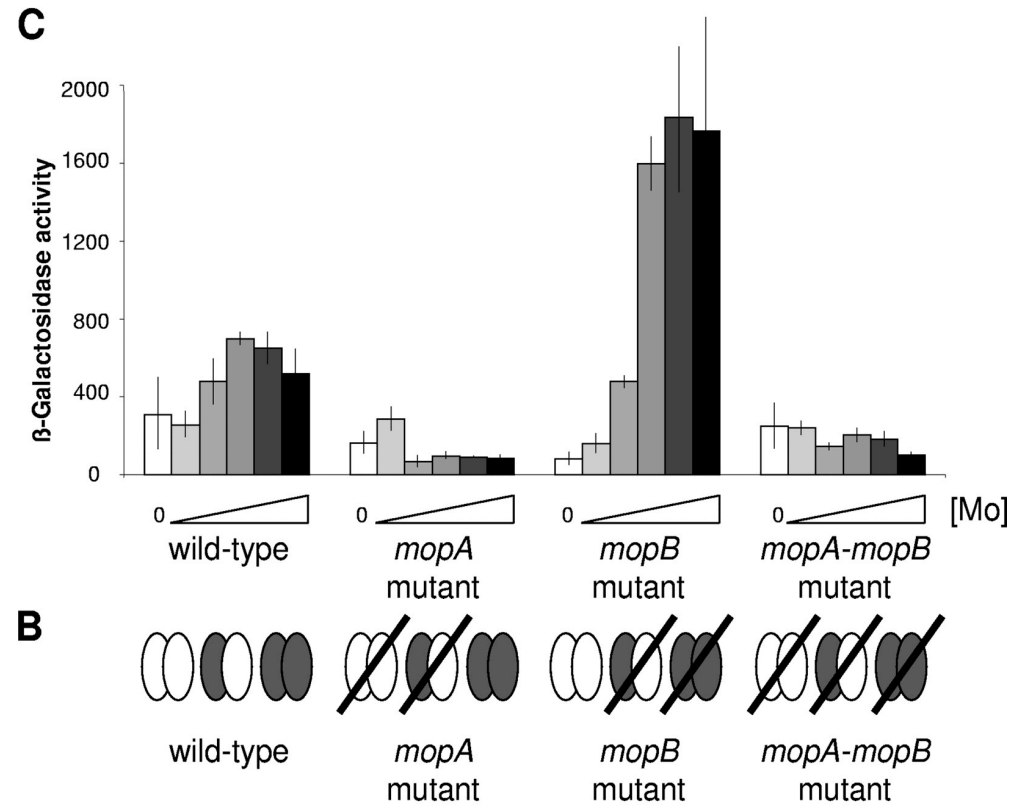
B



MopA-MopB heteromers

1. Can't bind *mop* promoter?
2. Have a lower affinity than MopA?
3. Can bind promoter but can not activate?

mop::lacZ



- 0
- 10nm
- 100nm
- 1µm
- 10µm
- 100µm

MopA is required for *mop* transcription

CONCLUSIONS

Rhodobacter capsulatus Encodes for 2 ModE-like Regulators

Unlike any other ModE Regulatory System

Could allow very specific regulation of Mo-Genes

MopA-MopB Ratios would change based on [Mo]