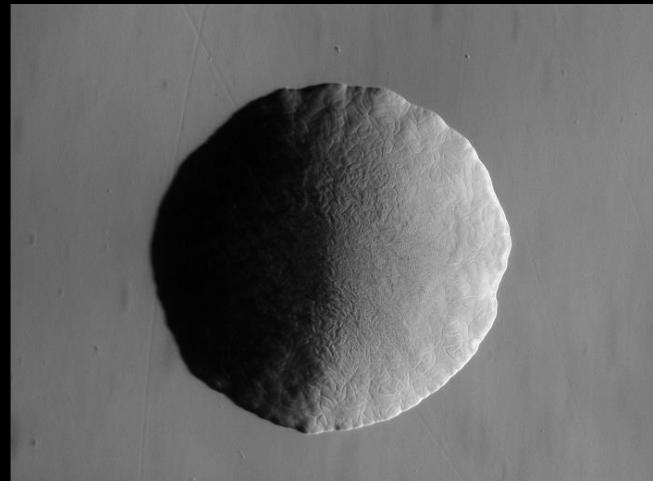
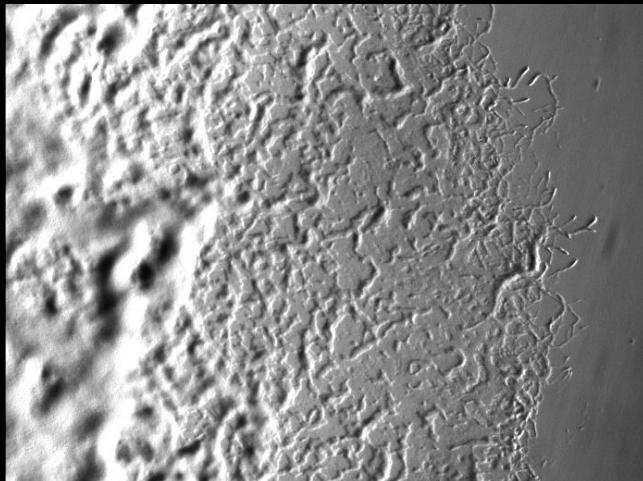


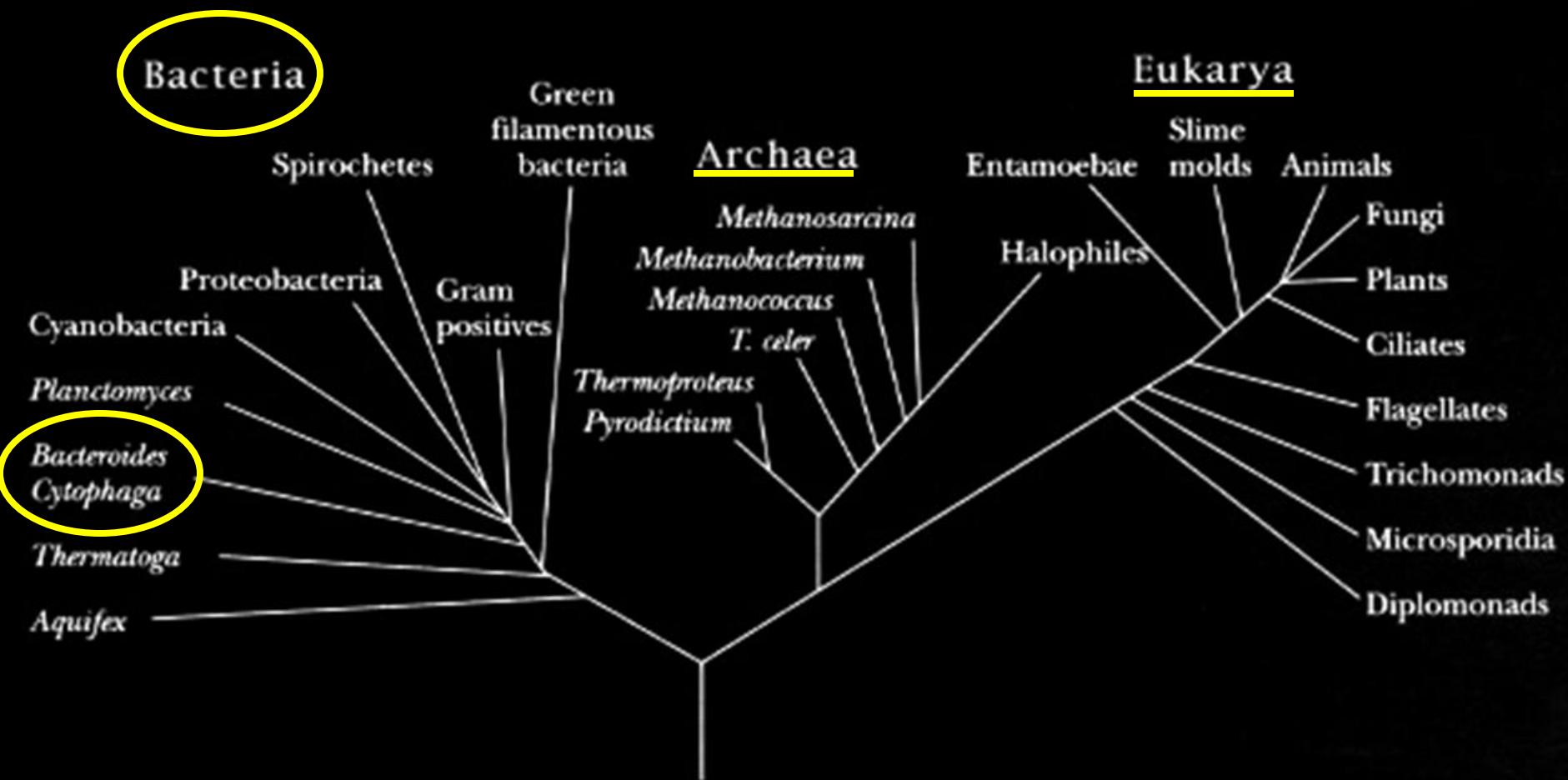
Mobile Cell Surface Adhesins and a Novel Protein Secretion System are Required for Gliding Motility in *Flavobacterium johnsoniae*

Ryan G. Rhodes

University of North Carolina Wilmington

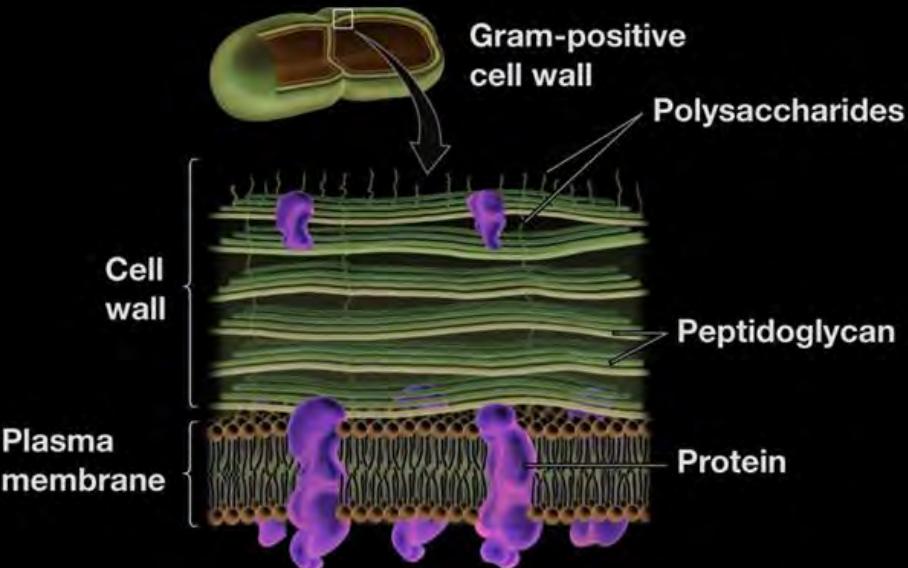


The Tree of Life



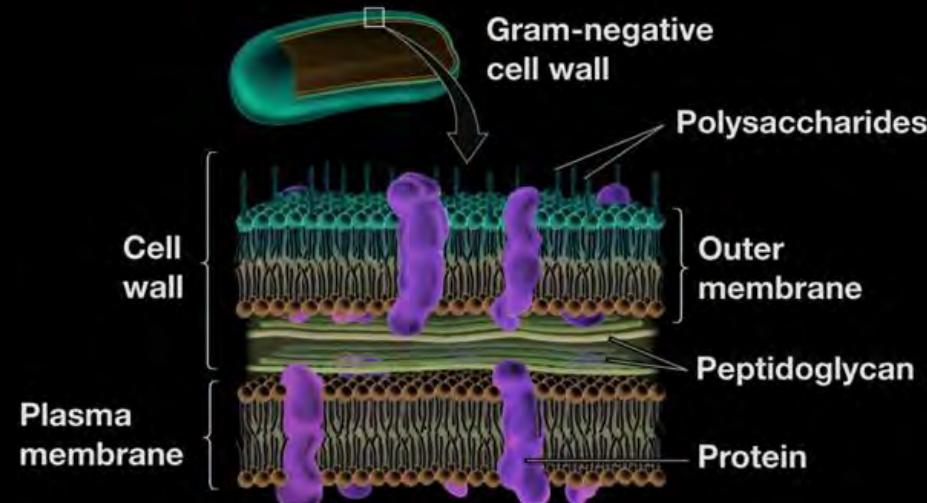
The Bacterial Cell Envelope

Gram-positive



Freeman. *Biological Science*. 3/e.

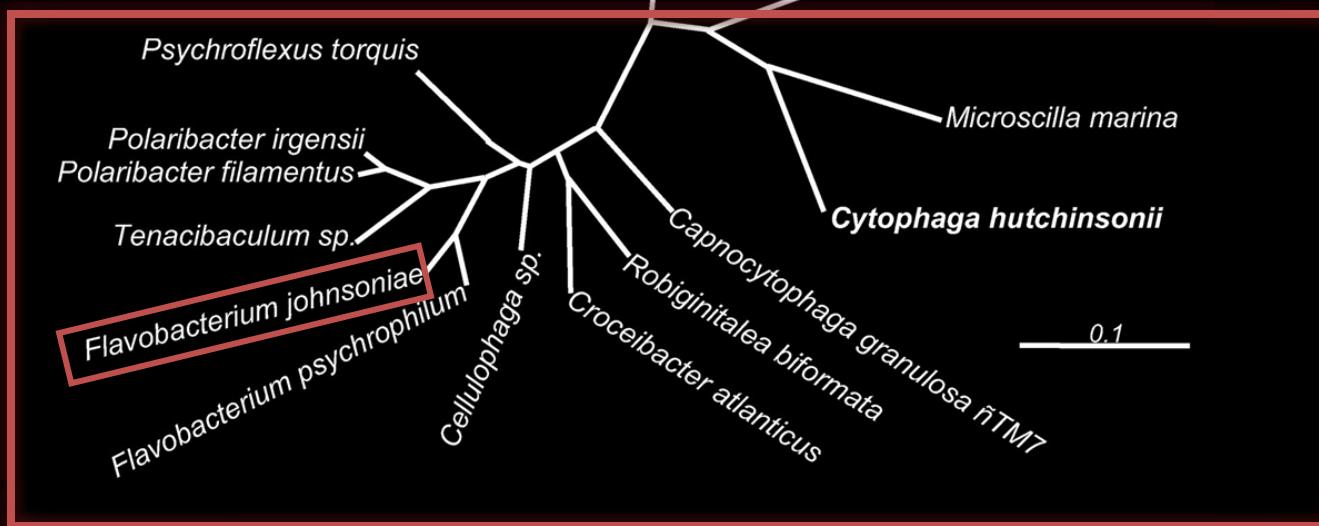
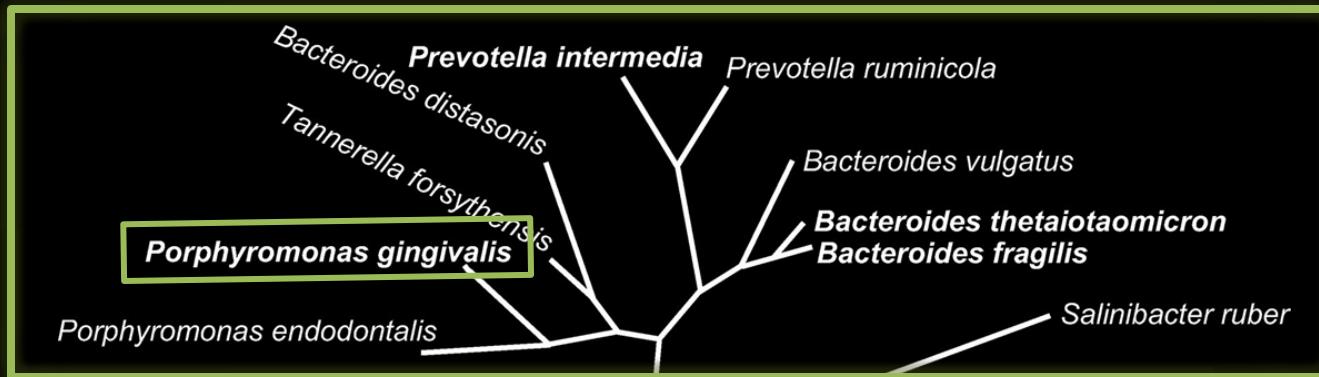
Gram-negative



Bacteroidetes are
Gram-negative bacteria

Bacteroidetes

Nonmotile

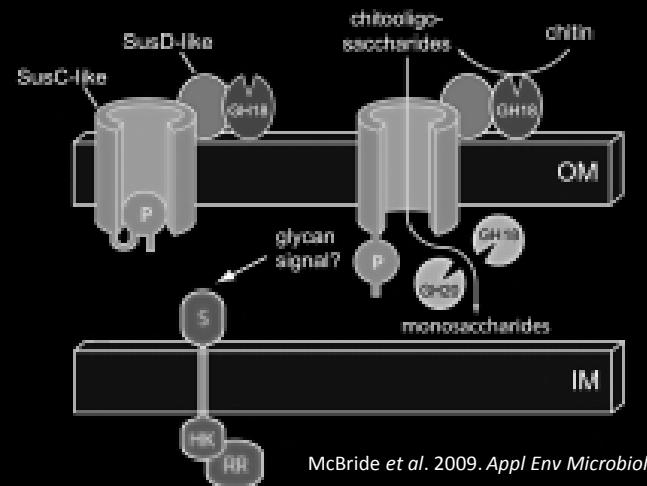
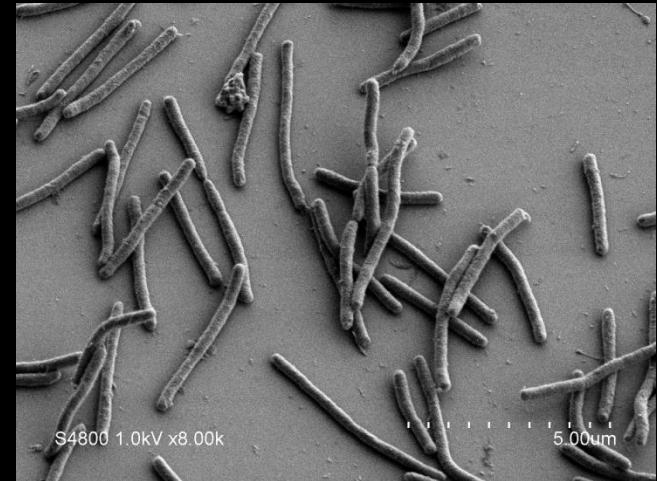


Motile – gliding motility

Xie et al. 2007. *Appl Environ Microbiol.*

Flavobacterium johnsoniae

- Gram-negative aerobic rod
- Member of the *Bacteroidetes*
- Ubiquitous in soil and freshwater habitats
- 6.1 Mbp sequenced genome
- Digests many polysaccharides
- Well characterized genetic system
- Exhibits gliding motility
- Newly described Type IX protein secretion

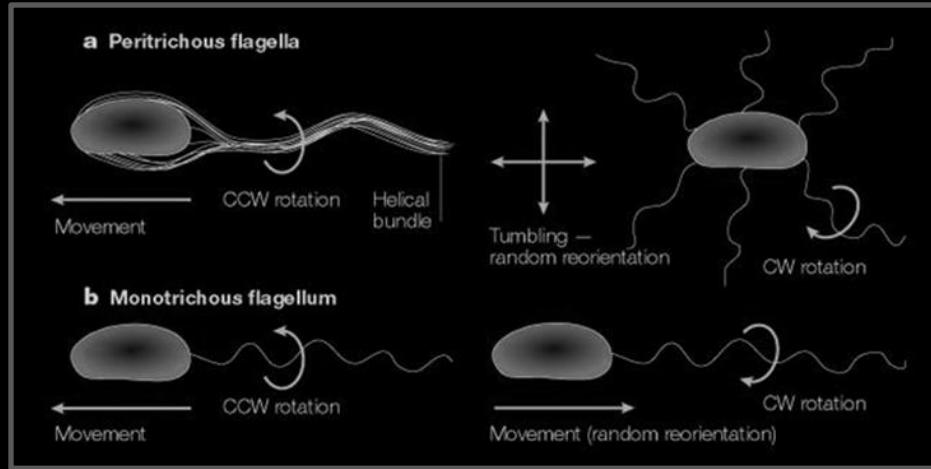


McBride et al. 2009. *Appl Env Microbiol.*

Well Studied Types of Bacterial Locomotion

Swimming Motility

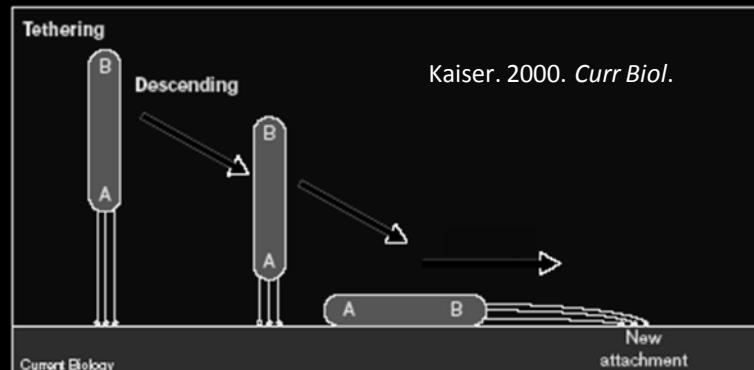
Flagella



Butler and Camilli. 2005. *Nat Rev Microbiol.*

Twitching Motility

Type IV pili



Kaiser. 2000. *Curr Biol.*

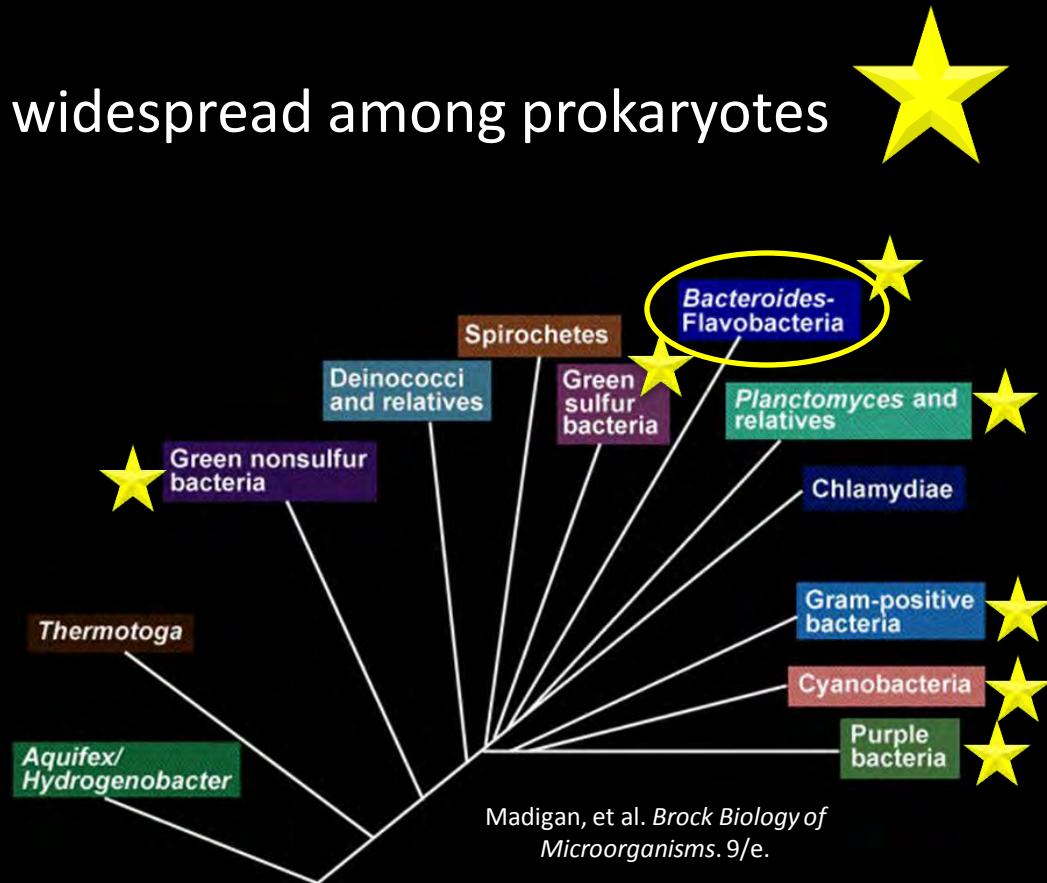
Gliding Motility

The smooth translocation of cells over a surface by an active process

Gliding motility is widespread among prokaryotes



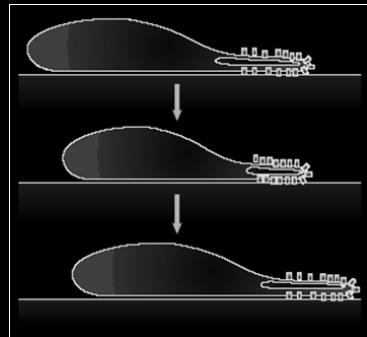
Bacterial Group	Representative genera of gliding bacteria
Cyanobacteria	<i>Oscillatoria, Spirulina, Anabaena, Phormidium</i> , many others
Cytophaga-Flavobacterium-group	<i>Flavobacterium^b, Cytophaga, Saprospira, Flexibacter</i> , many others
δ Proteobacteria	<i>Myxococcus, Stigmatella, Chondromyces, Desulfonema</i> , many others
β Proteobacteria	<i>Vitreoscilla, Simonsiella</i>
γ Proteobacteria	<i>Lysobacter, Beggiatoa, Leucothrix, Thiothrix</i>
Green nonsulfur bacteria	<i>Chloroflexus, Herpetosiphon, Heliothrix</i>
Green sulfur bacteria	<i>Chloroherpeton</i>
Planctomyces	<i>Isosphaera</i>
Gram-positive bacteria ^c	<i>Heliobacterium, Filibacter, Mycoplasma^d</i>



Variety of gliding mechanisms

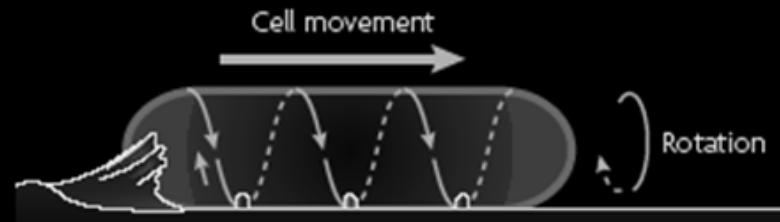
Examples of Gliding Motility

Mycoplasma pneumoniae



Jarrell and McBride. 2008. *Nat Rev Microbiol.*

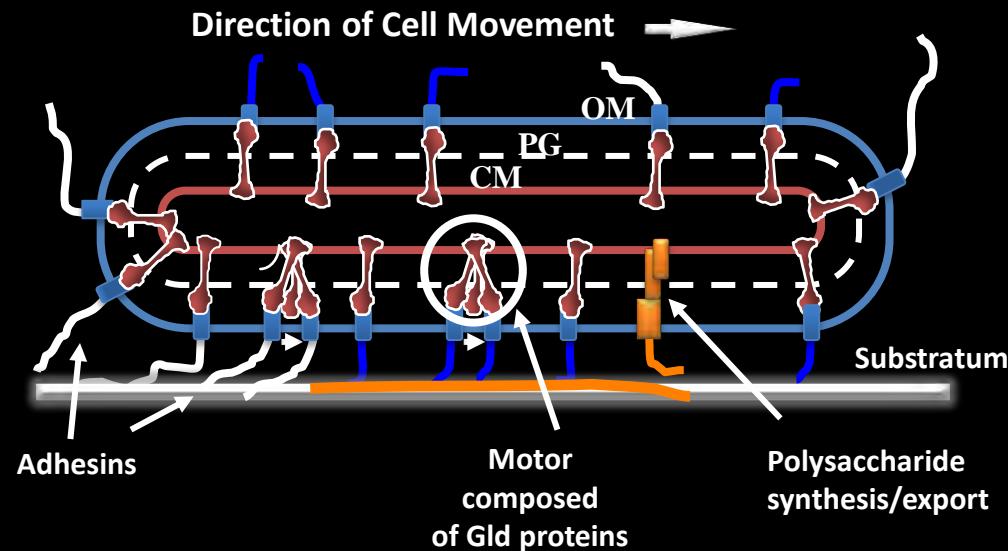
Myxococcus xanthus



Motors maintain fixed positions with respect to the substratum and push the cell body forward

Jarrell and McBride. 2008. *Nat Rev Microbiol.*

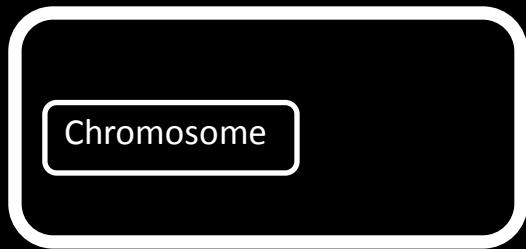
Flavobacterium johnsoniae



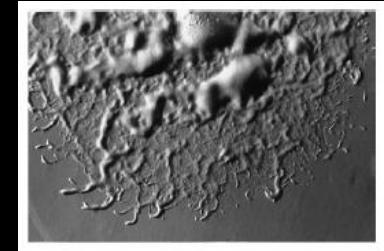
F. johnsoniae Gliding



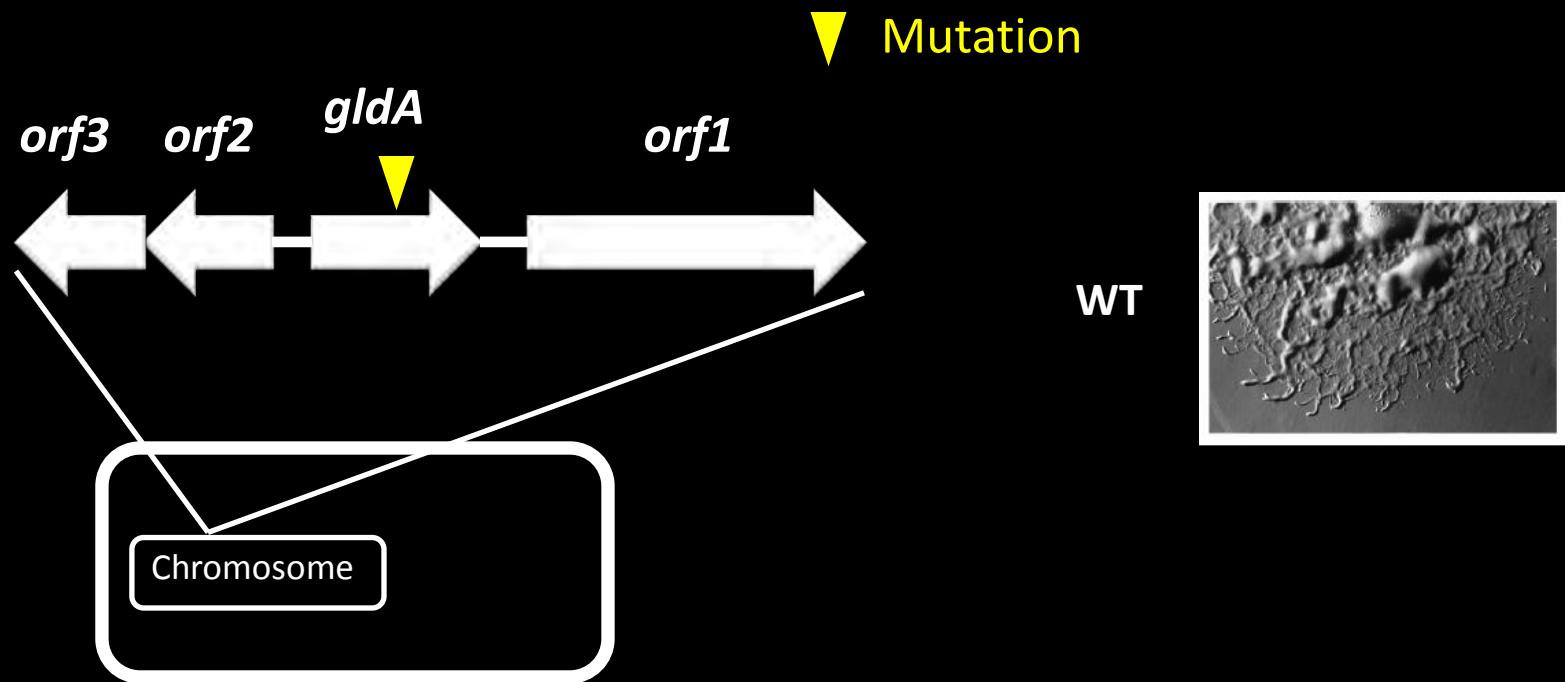
Identification of Motility Genes: *gldA*



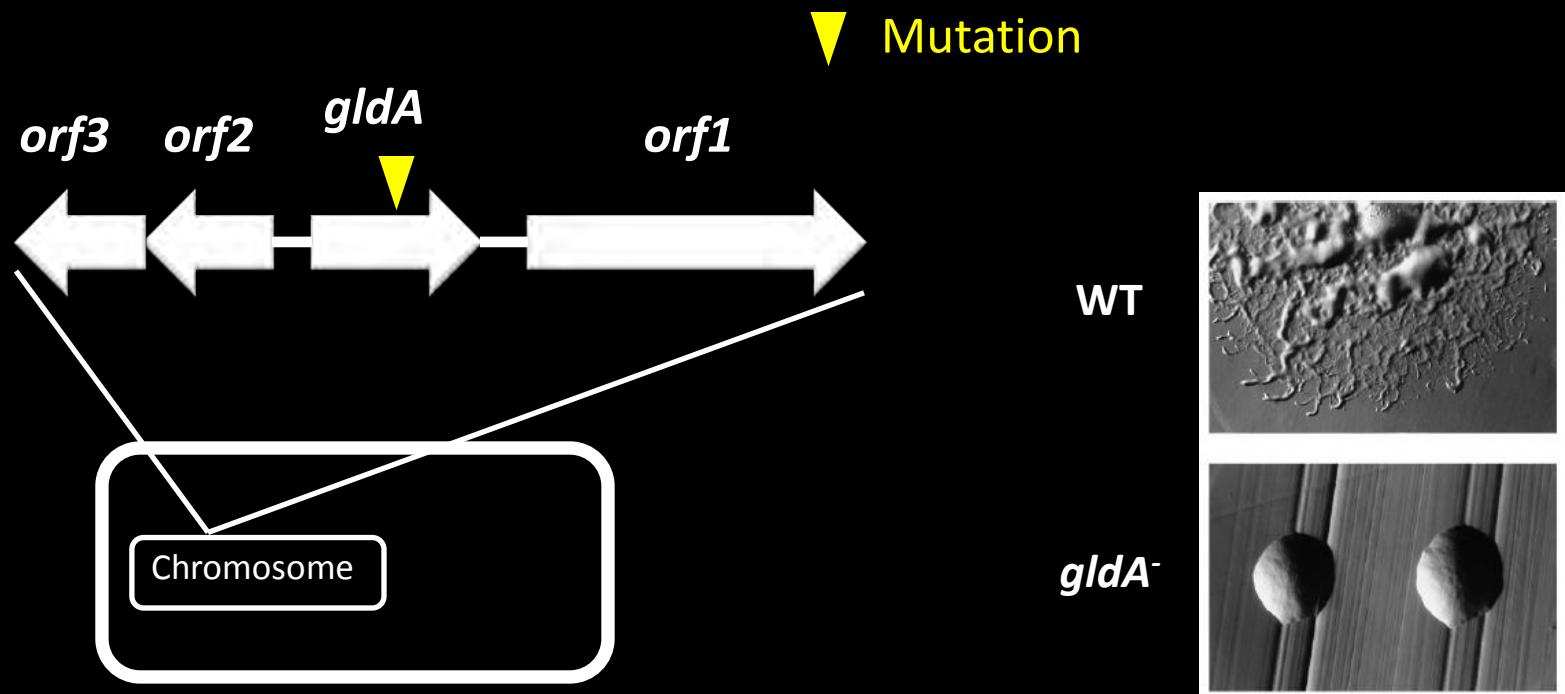
WT



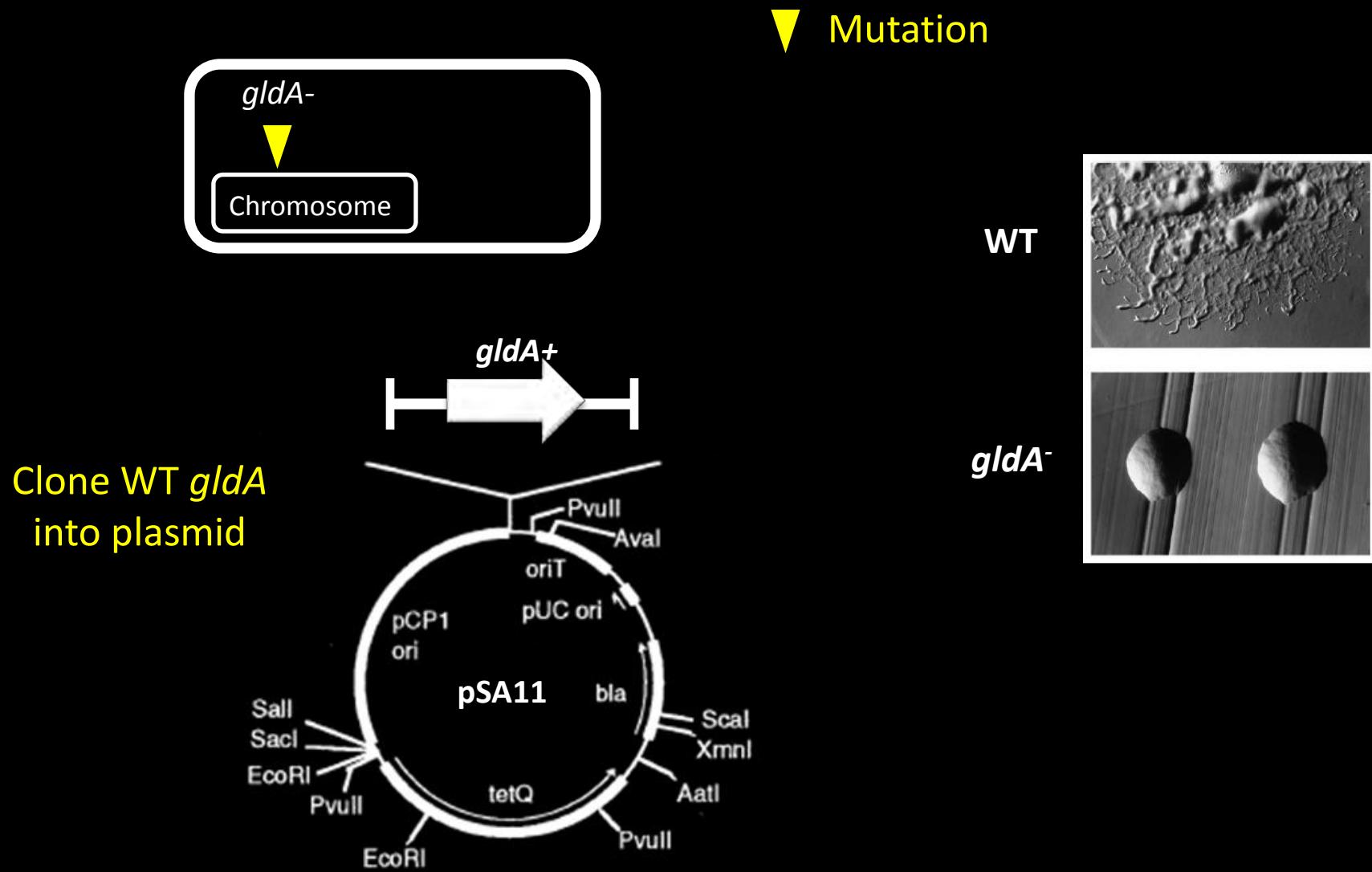
Identification of Motility Genes: *gldA*



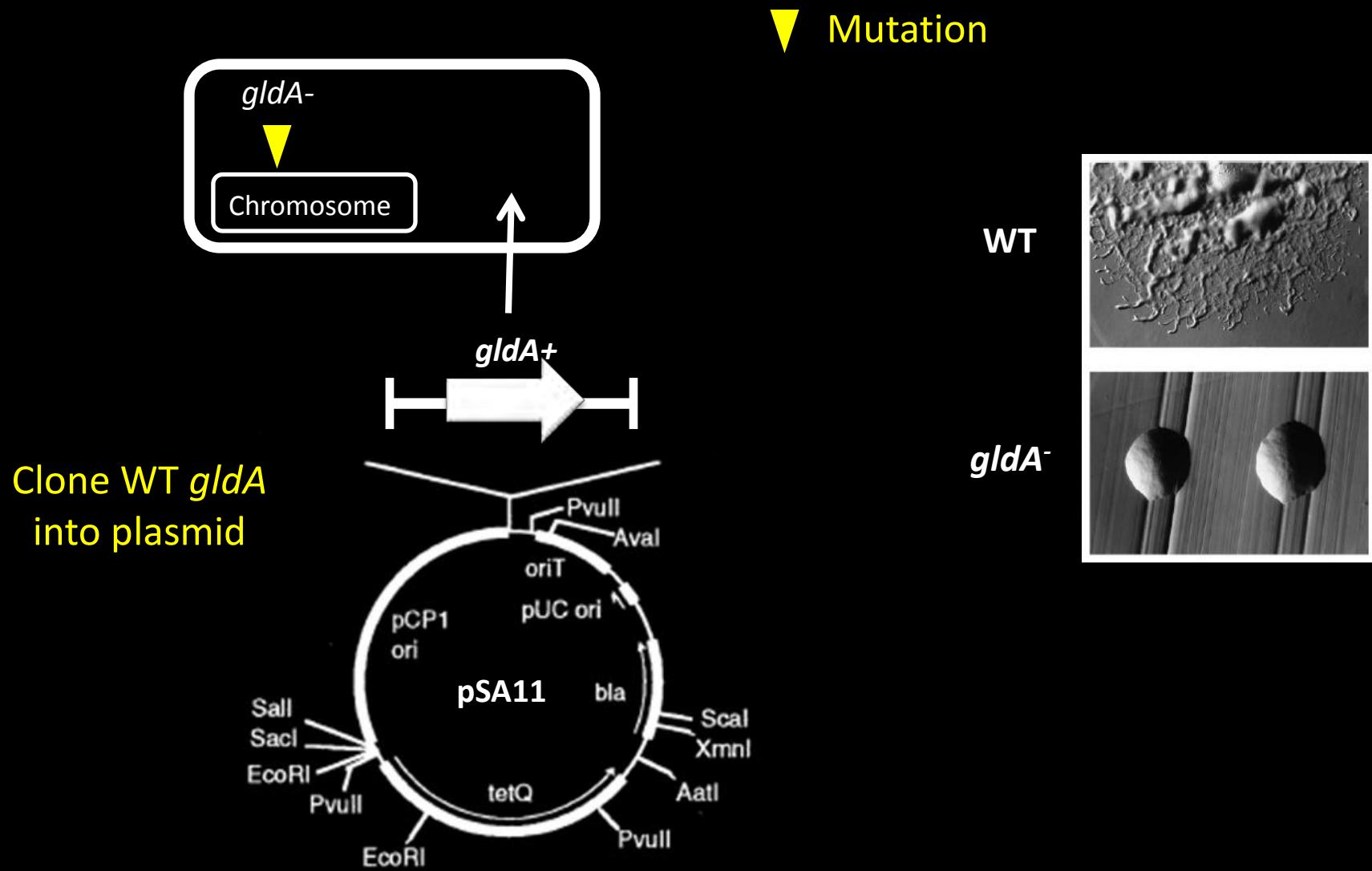
Identification of Motility Genes: *gldA*



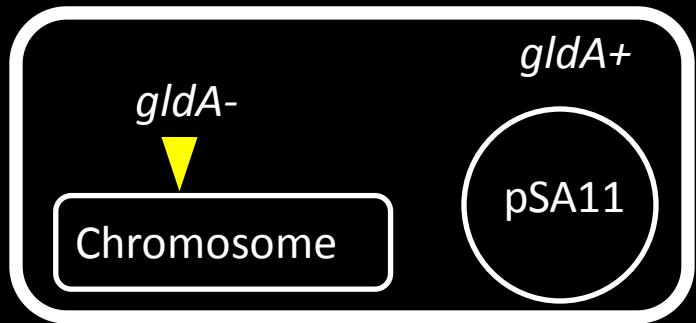
Identification of Motility Genes: *gldA*



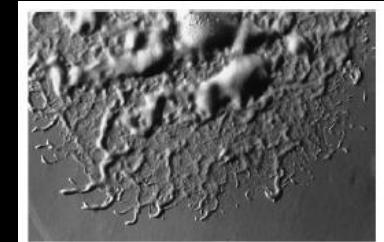
Identification of Motility Genes: *gldA*



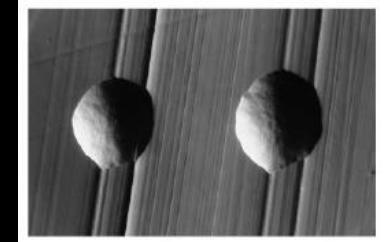
Identification of Motility Genes: *gldA*



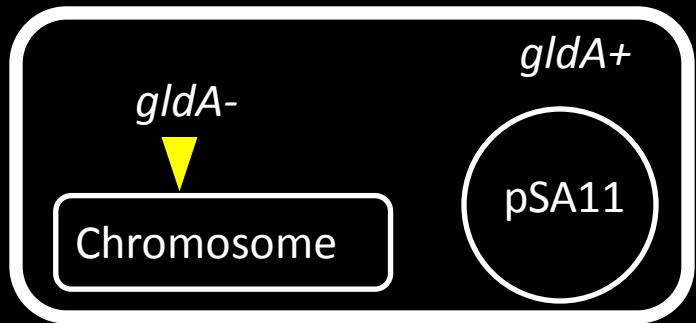
WT



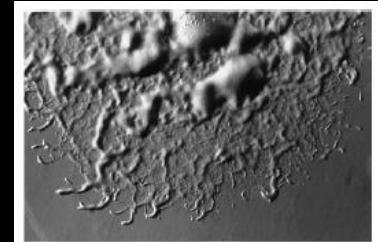
gldA⁻



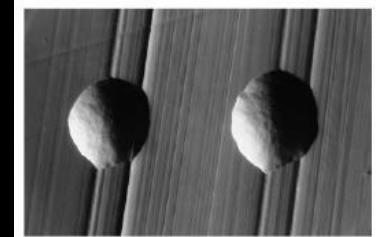
Identification of Motility Genes: *gldA*



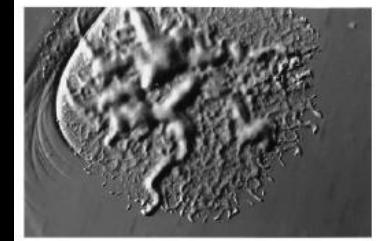
WT



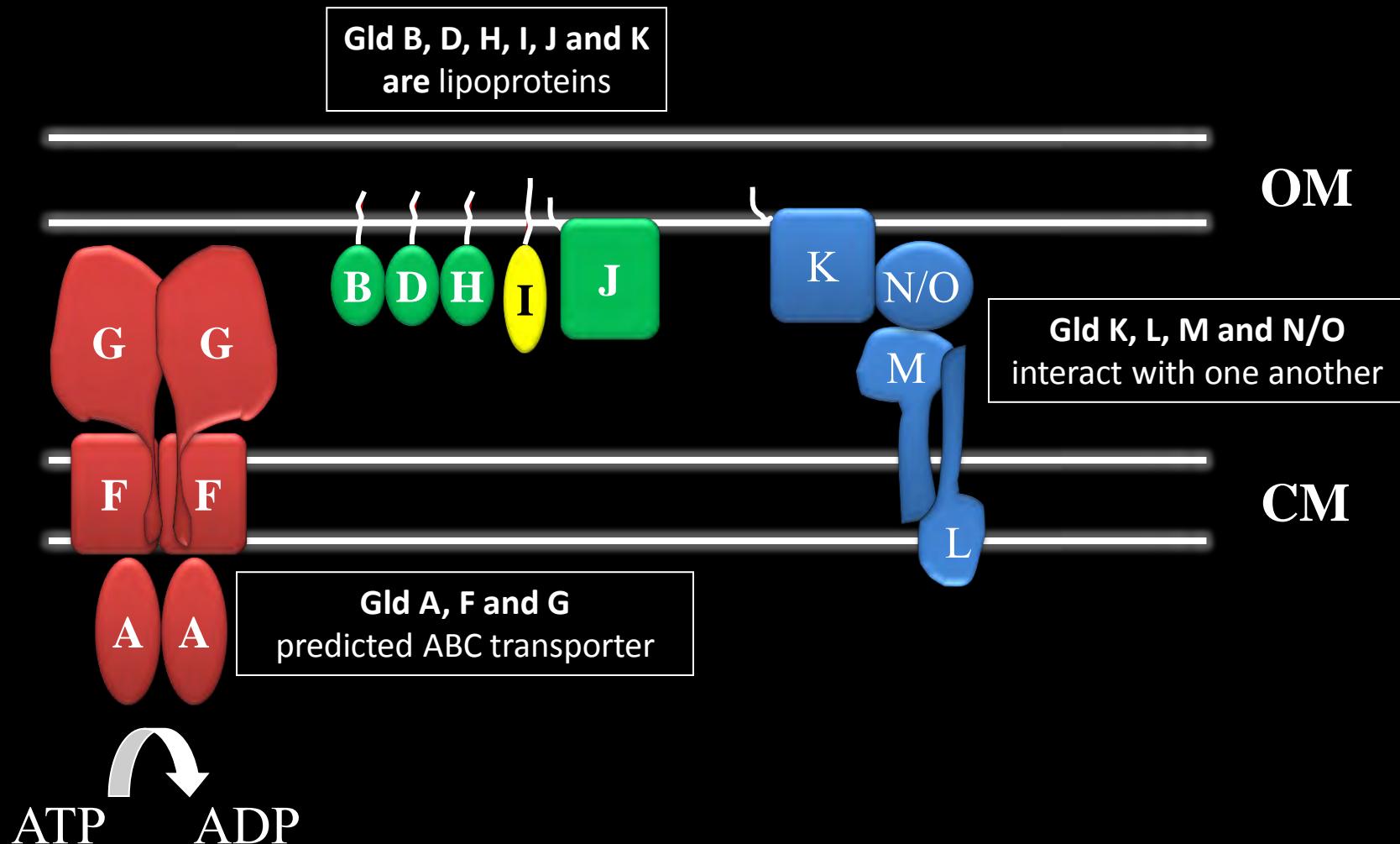
gldA-



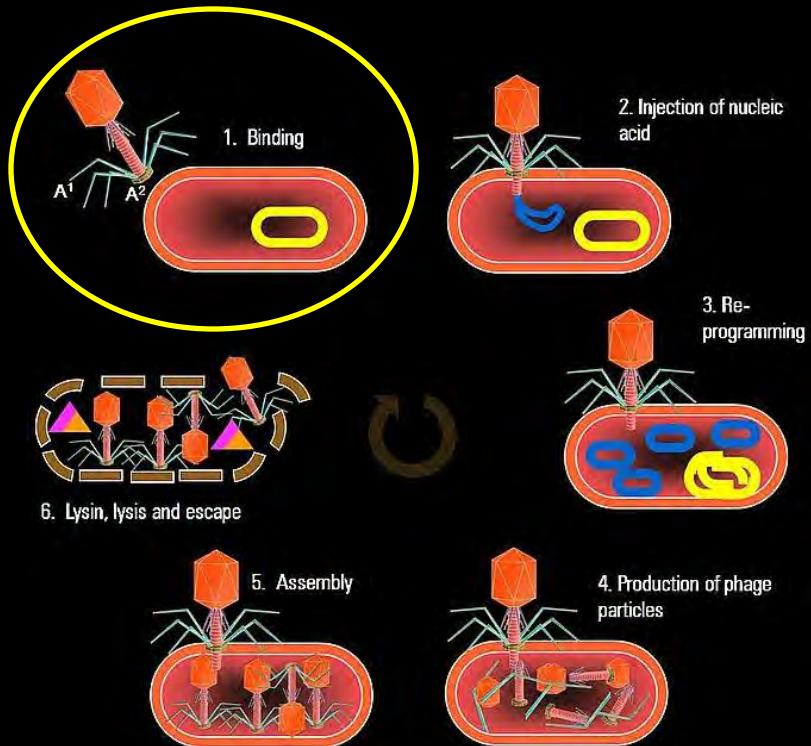
gldA-/gldA+



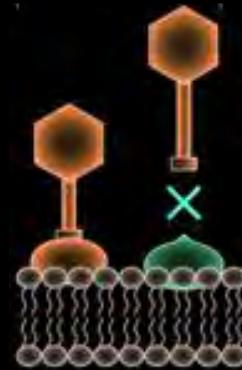
F. Johnsoniae Gld Proteins



gld Phenotypes: Phage Sensitivity



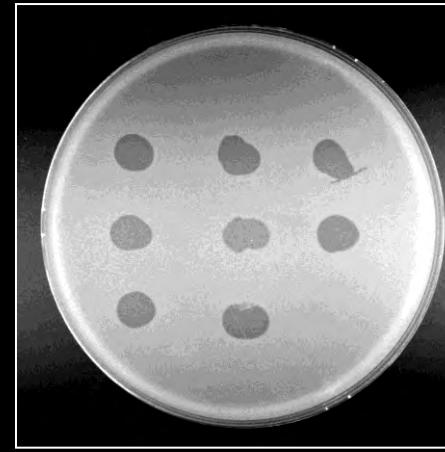
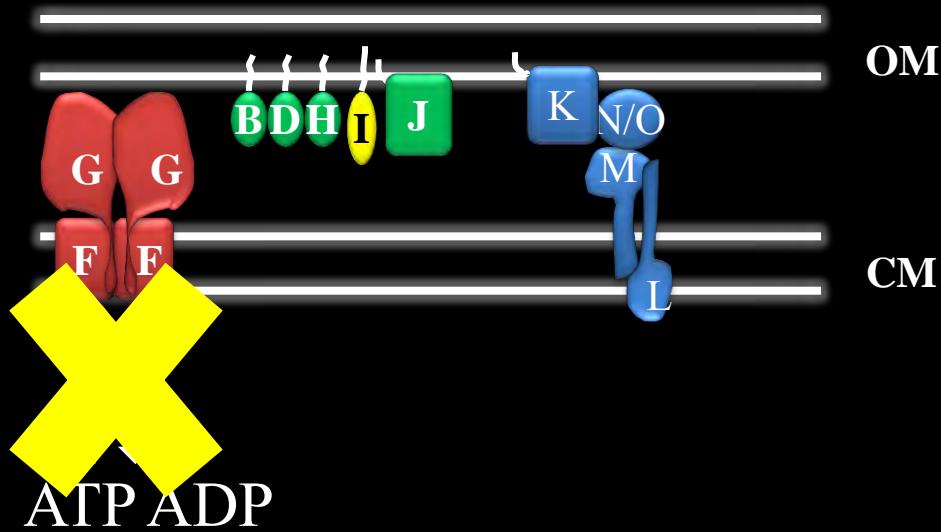
<http://www.hyglos.de>



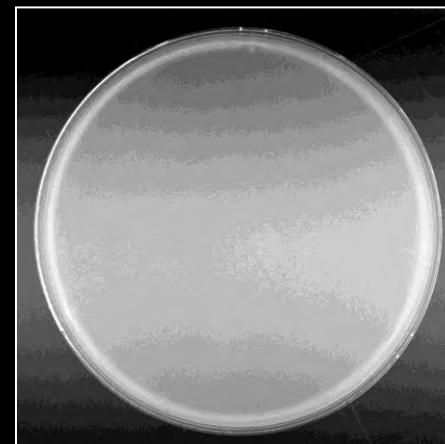
OM

<http://www.nature.com/nrmicro/journal/v8/n5/>

gld Phenotypes: Phage Sensitivity



WT



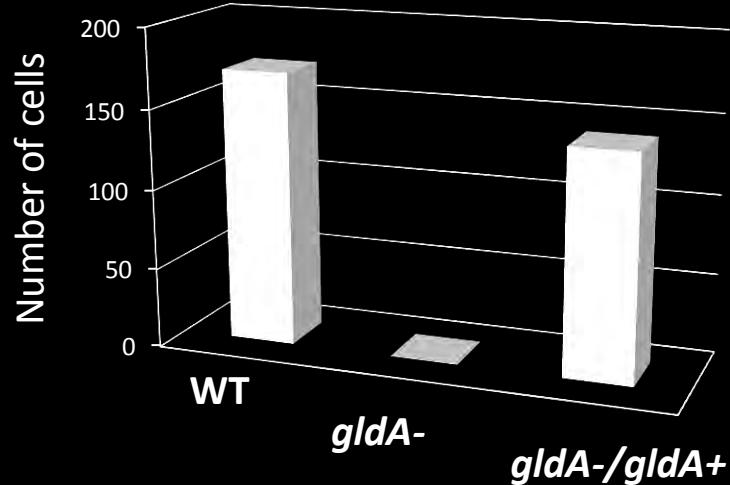
$\Delta gldA$

gld Phenotypes: Chitinase and Attachment

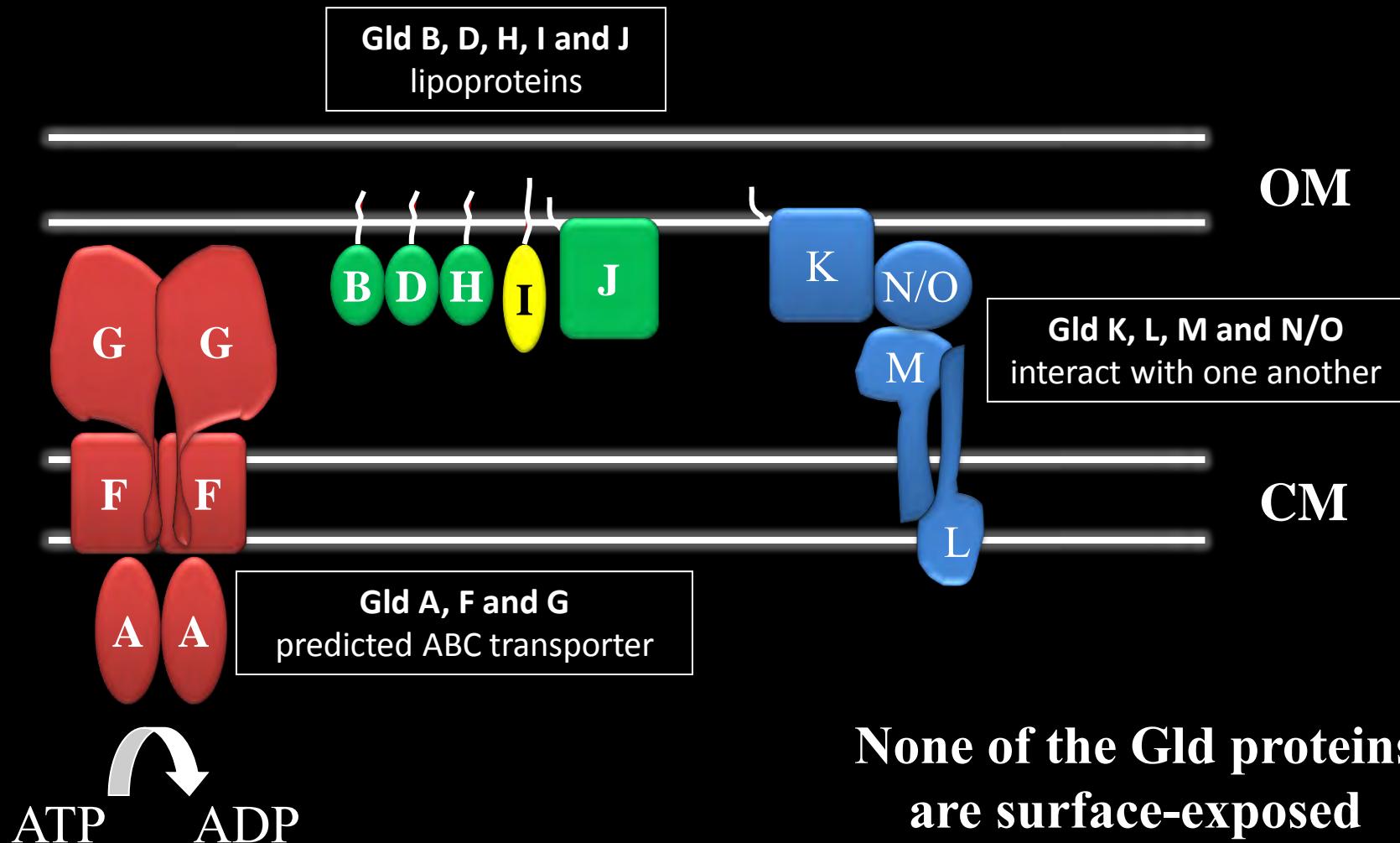
Chitin utilization



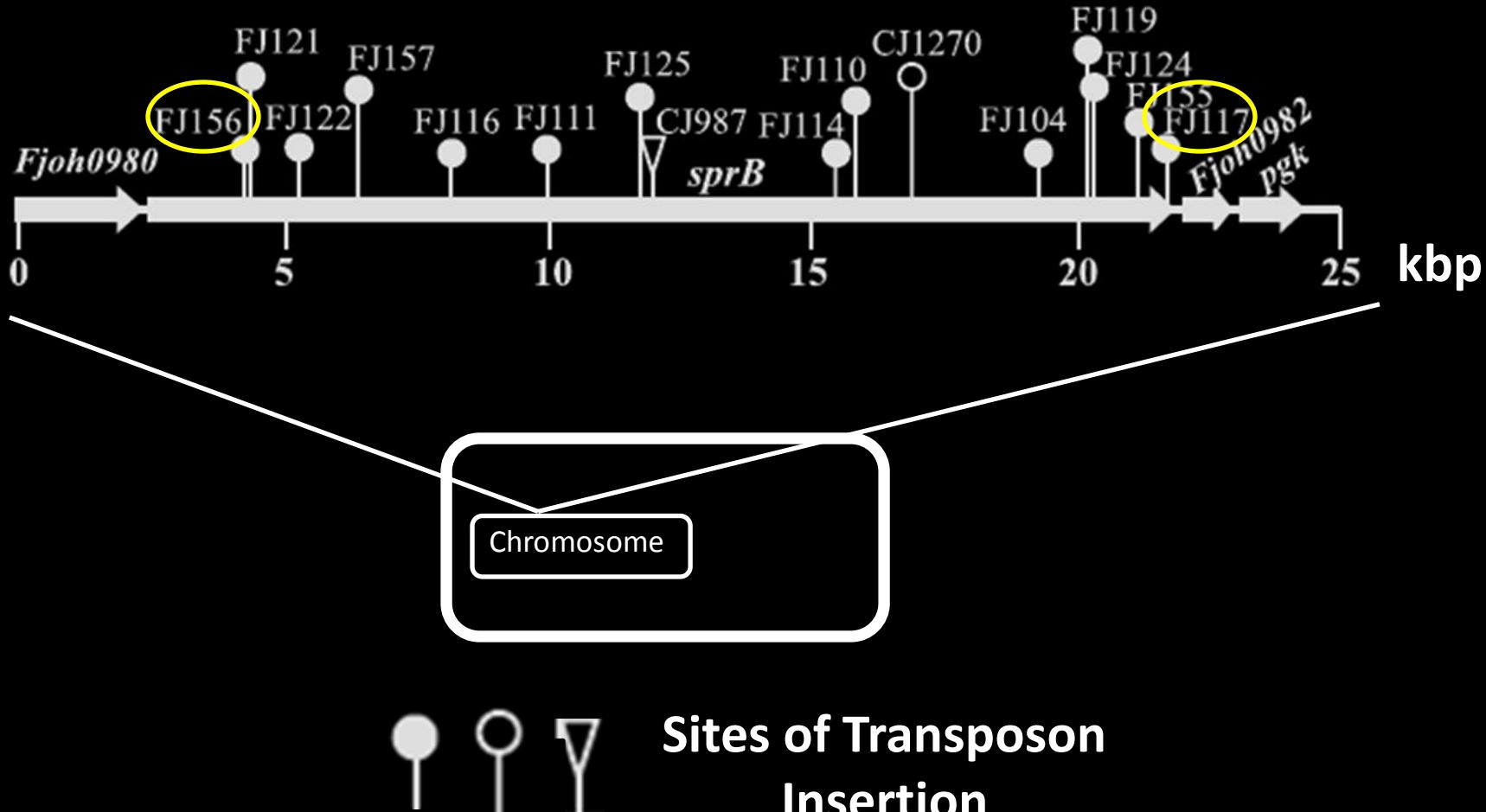
Attachment to Glass



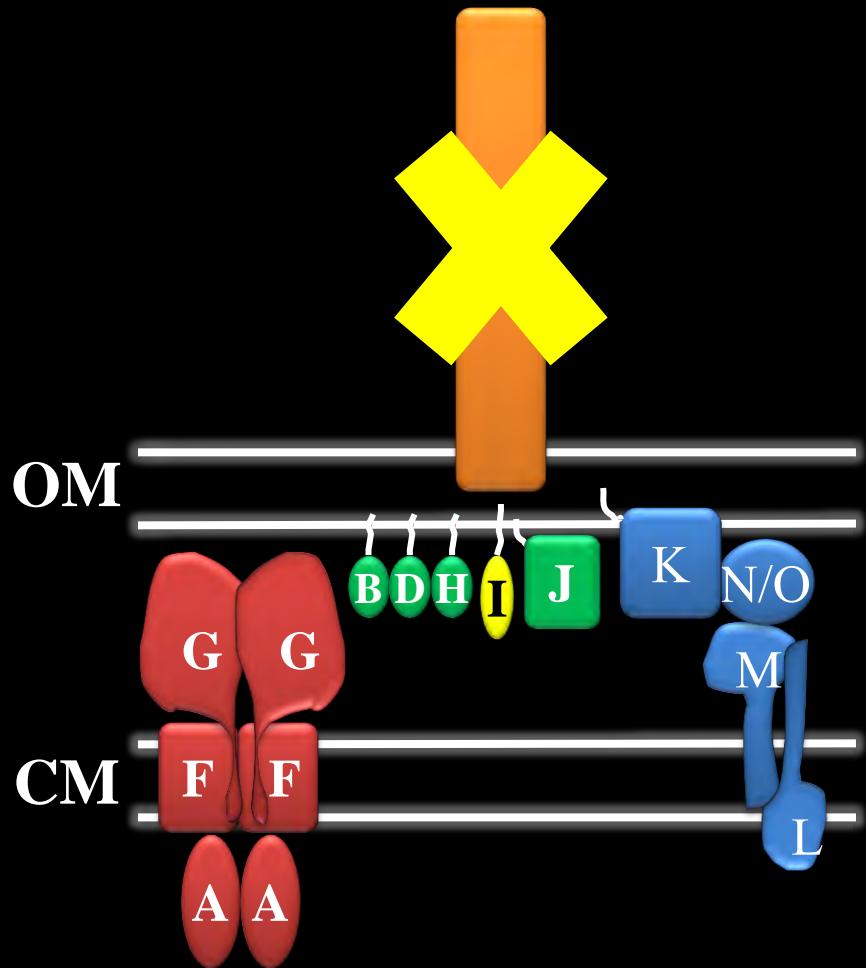
F. Johnsoniae Gld Proteins



Mutation in Surface Exposed *sprB*

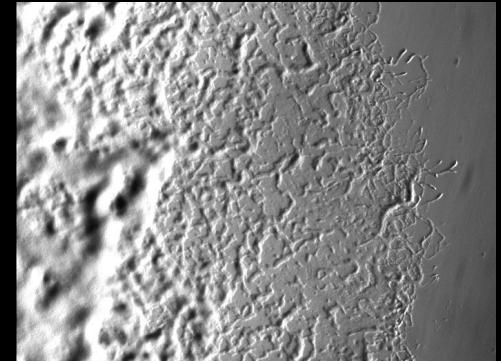


Properties of *sprB* Mutant

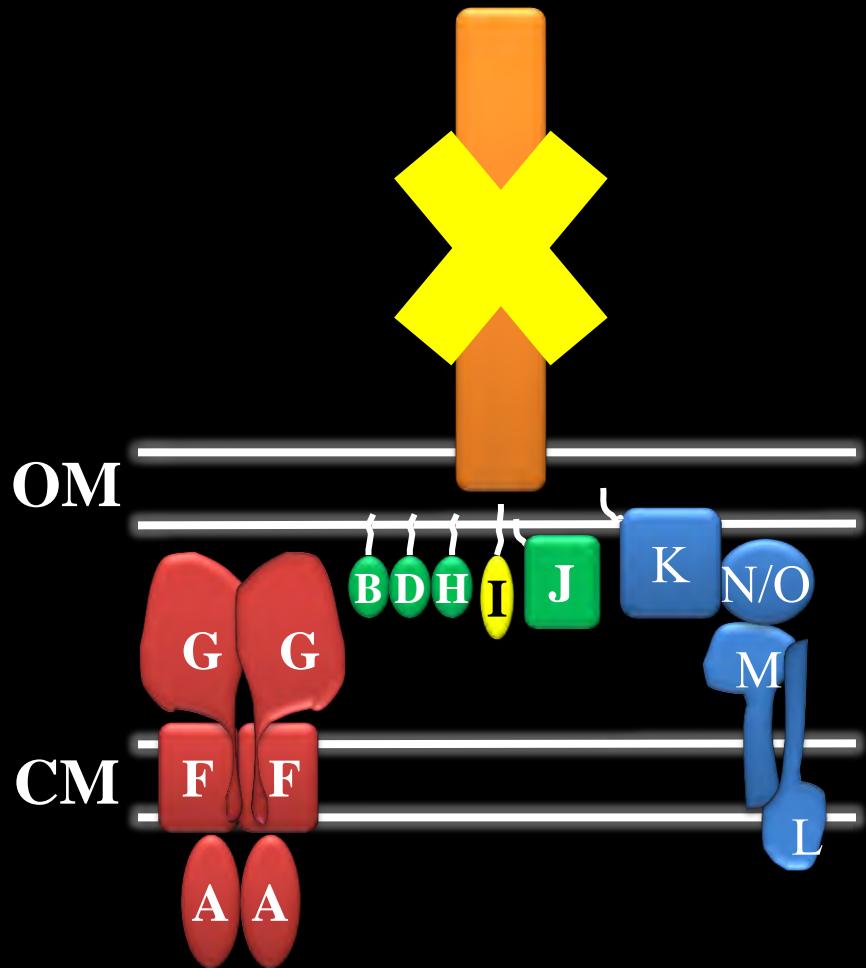


Spreading on Agar

WT

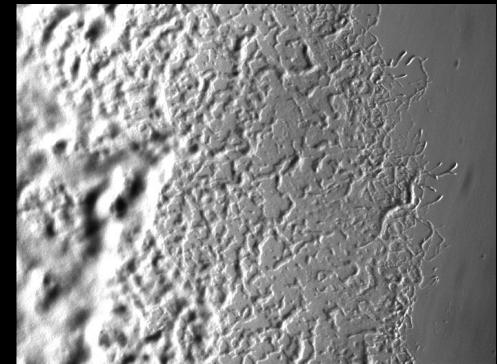


Properties of *sprB* Mutant



Spreading on Agar

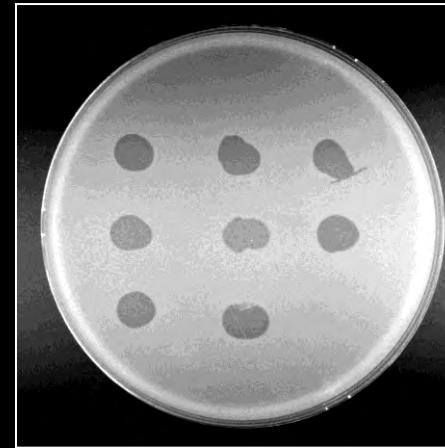
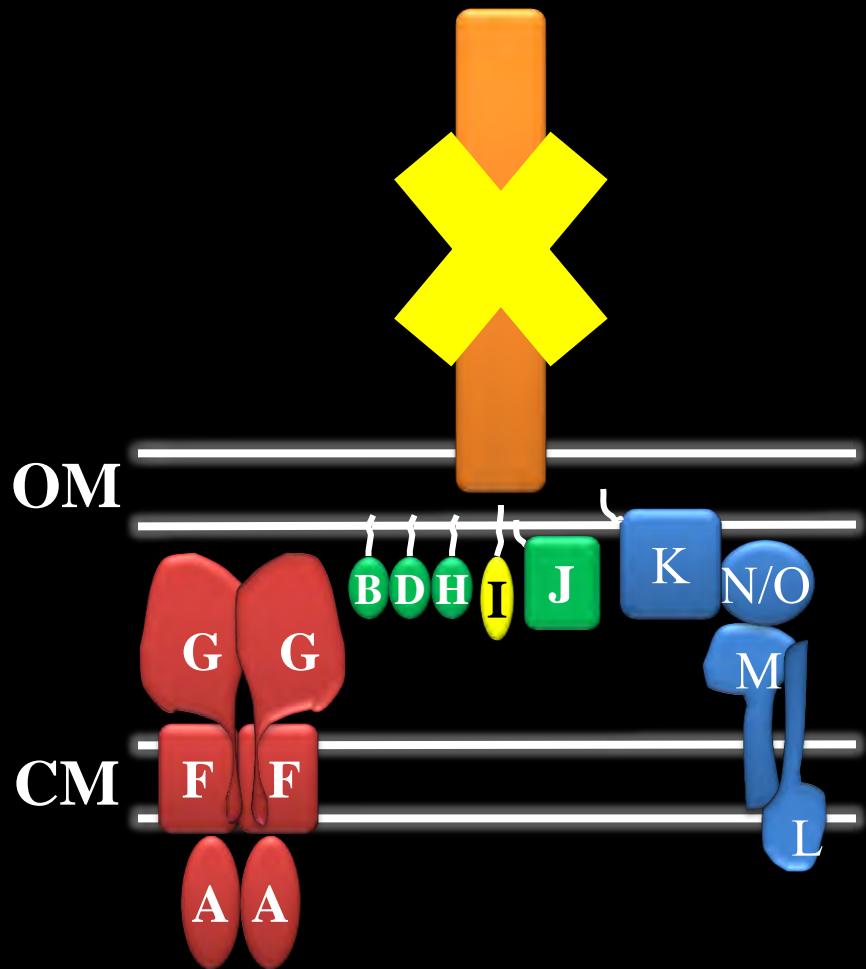
WT



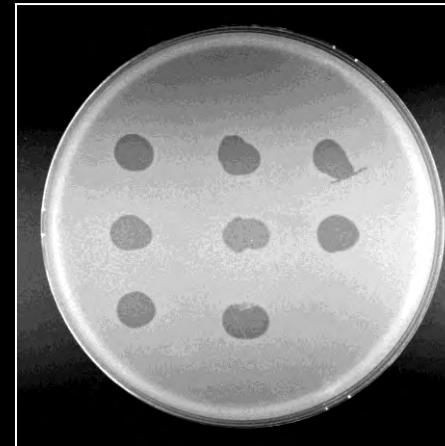
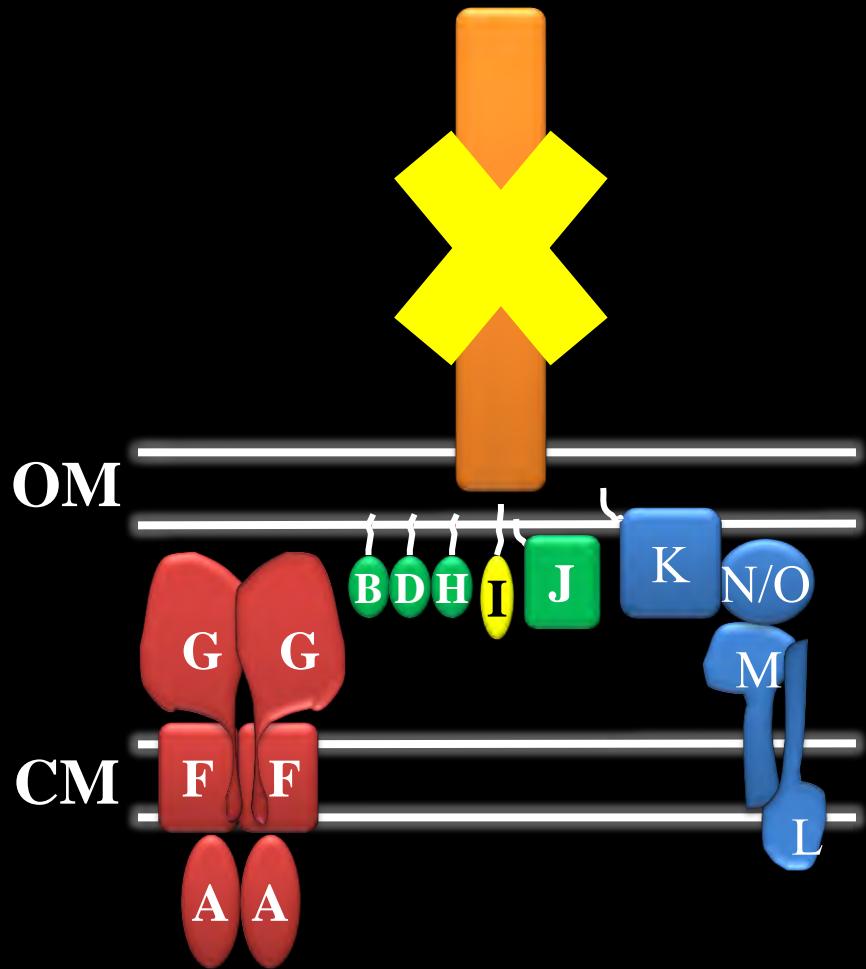
$\Delta sprB$



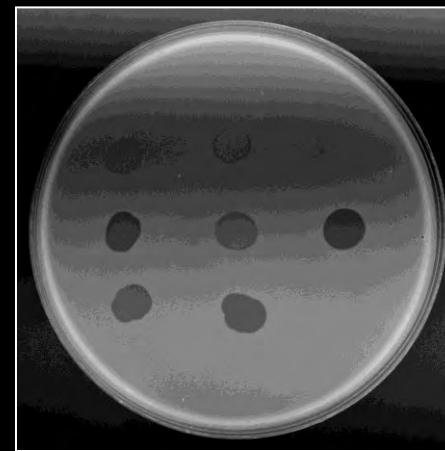
Properties of *sprB* Mutant



Properties of *sprB* Mutant



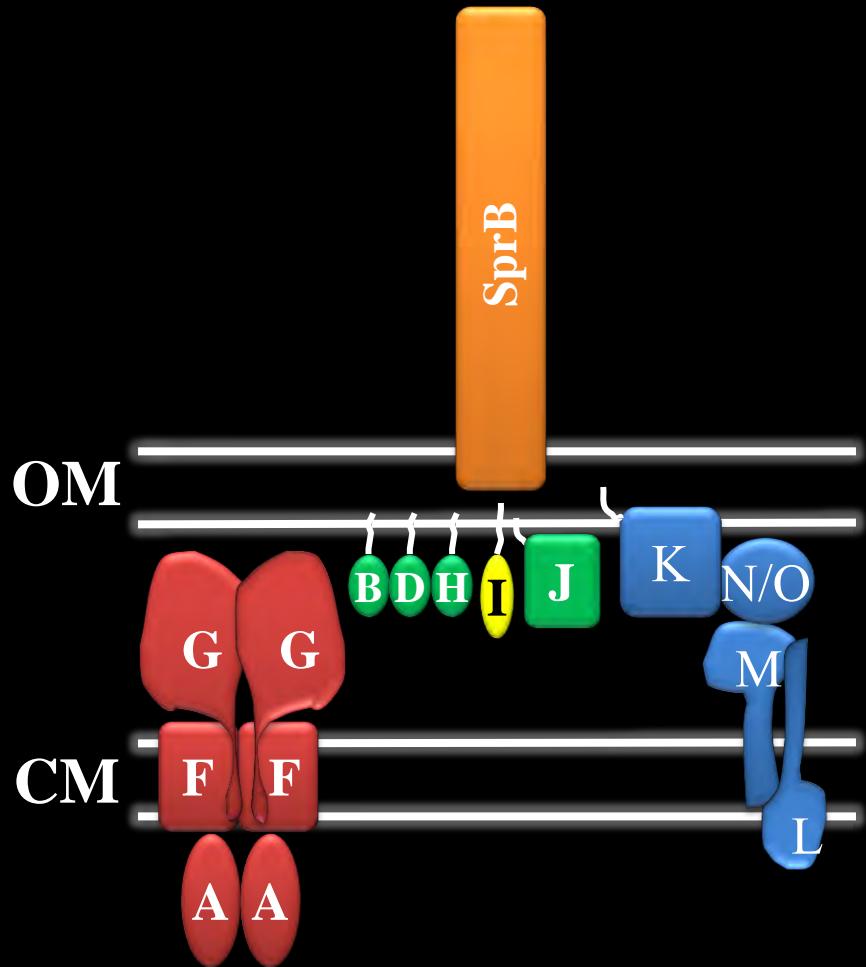
WT



$\Delta sprB$

Nelson, Bollampalli and McBride. 2008. *J Bacteriol.*

Properties of *sprB* Mutant

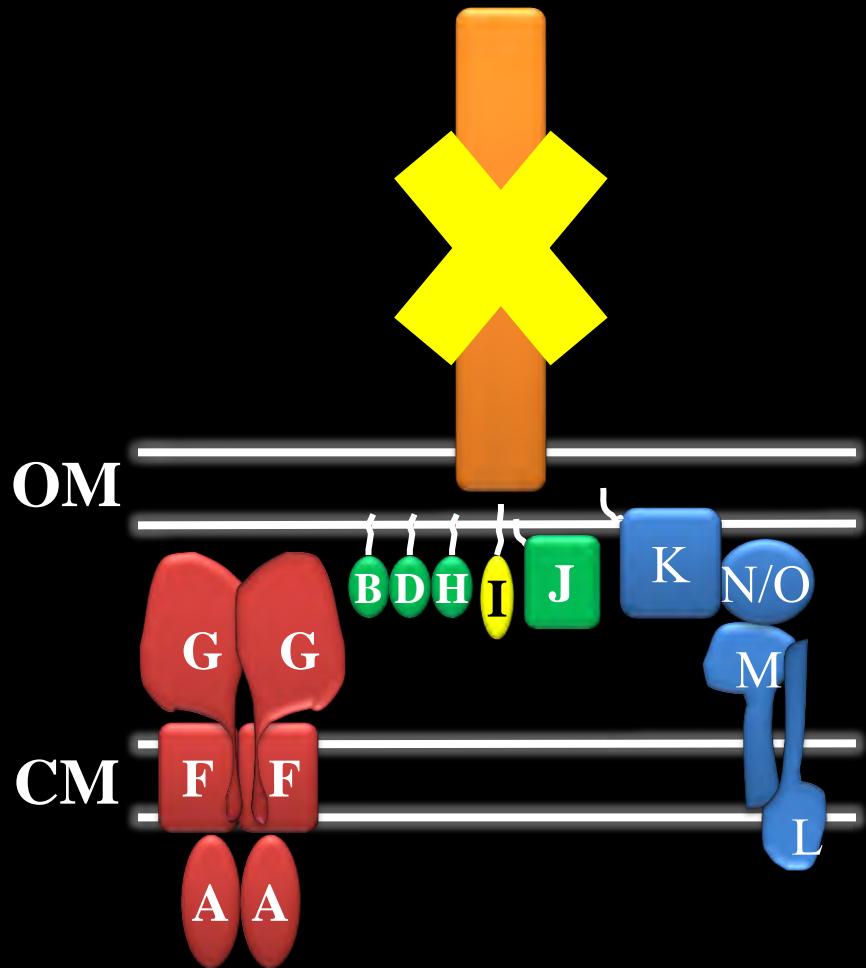


Gliding on Glass

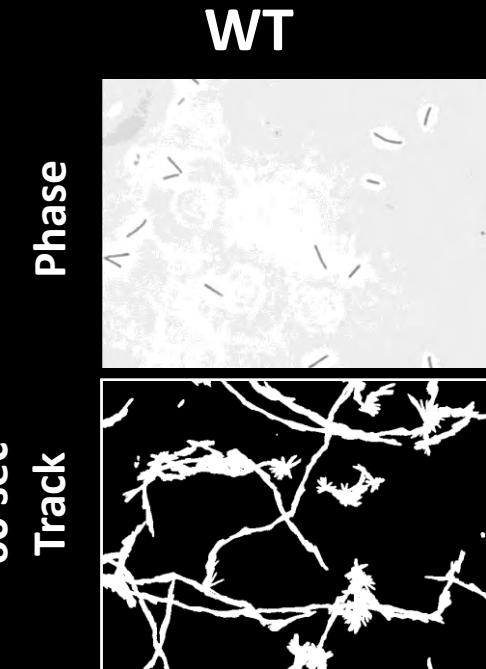
WT



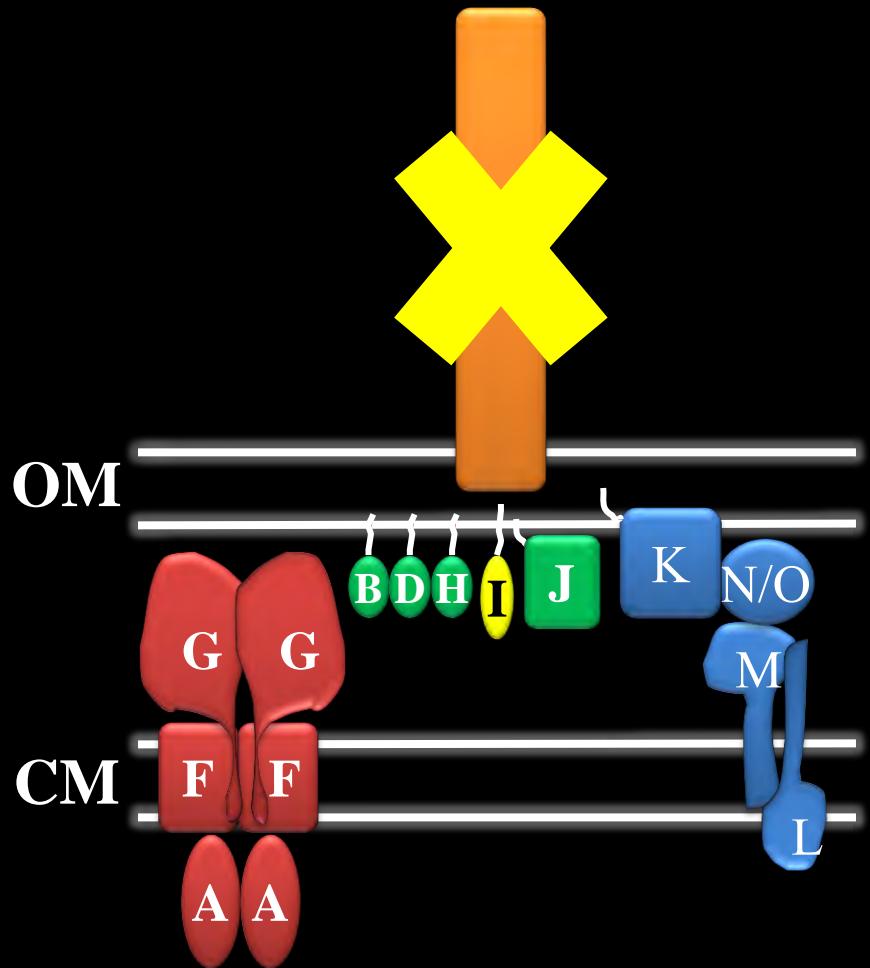
Properties of *sprB* Mutant



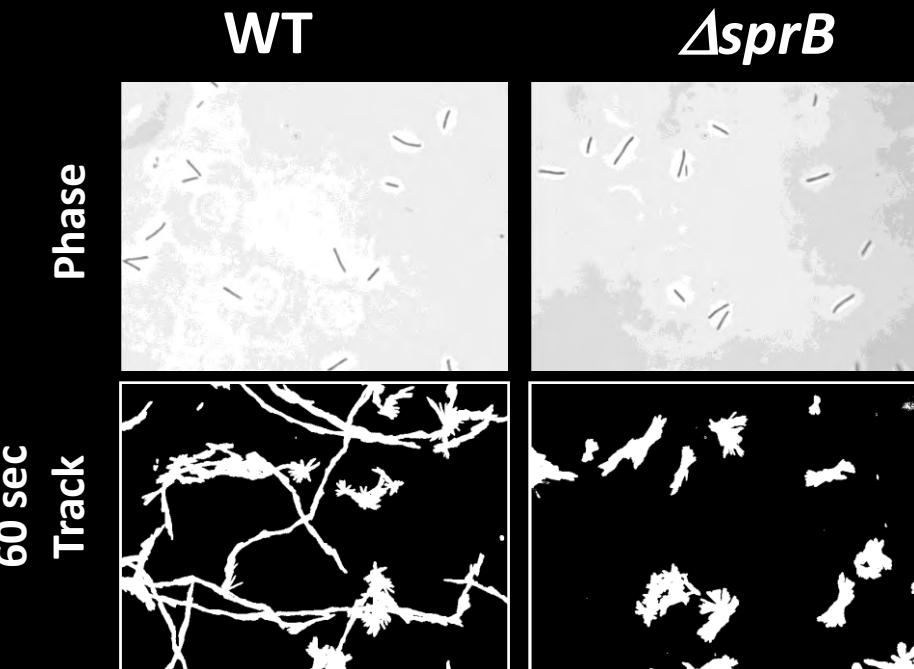
Gliding on Glass



Properties of *sprB* Mutant

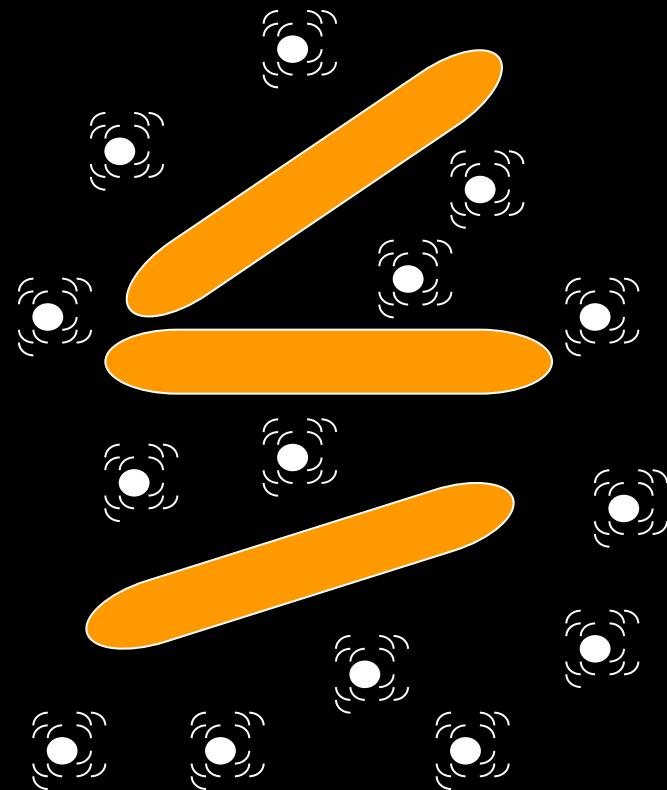


Gliding on Glass

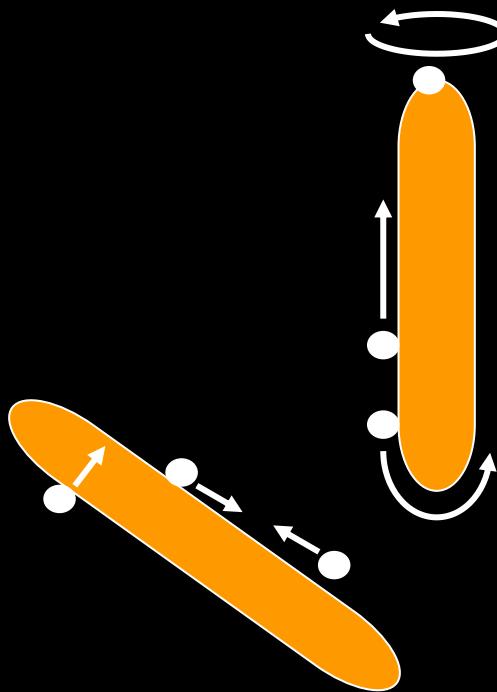


Bead Binding and Movement

WT cells with Protein G coated latex spheres

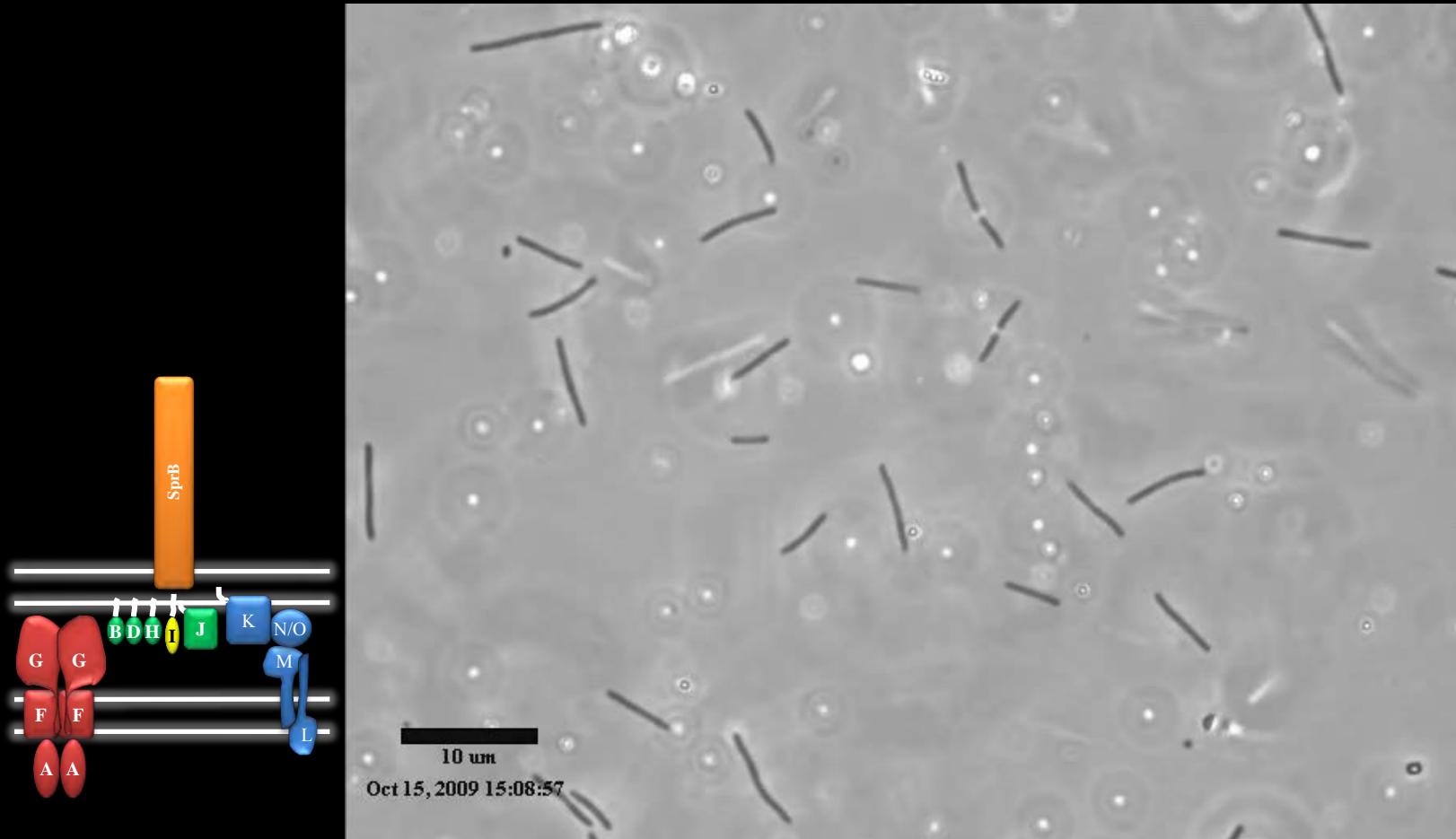


WT cells with Protein G coated latex spheres and Anti-SprB



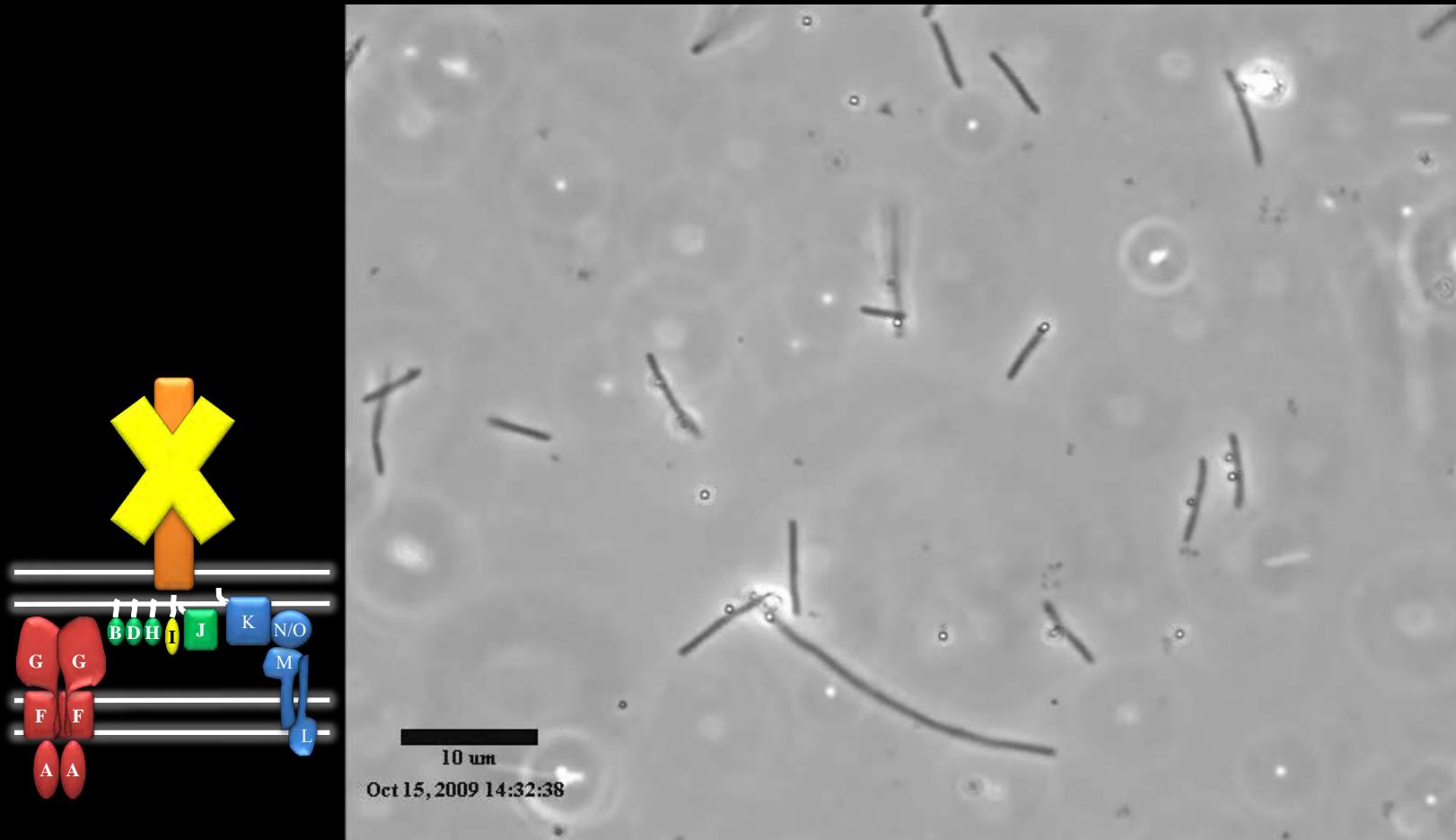
Bead Binding and Movement

WT Cells + Beads (No Ab)



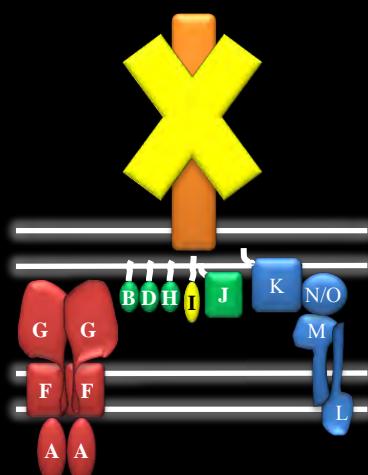
Bead Binding and Movement

WT Cells + Beads + Anti-SprB



Bead Binding and Movement

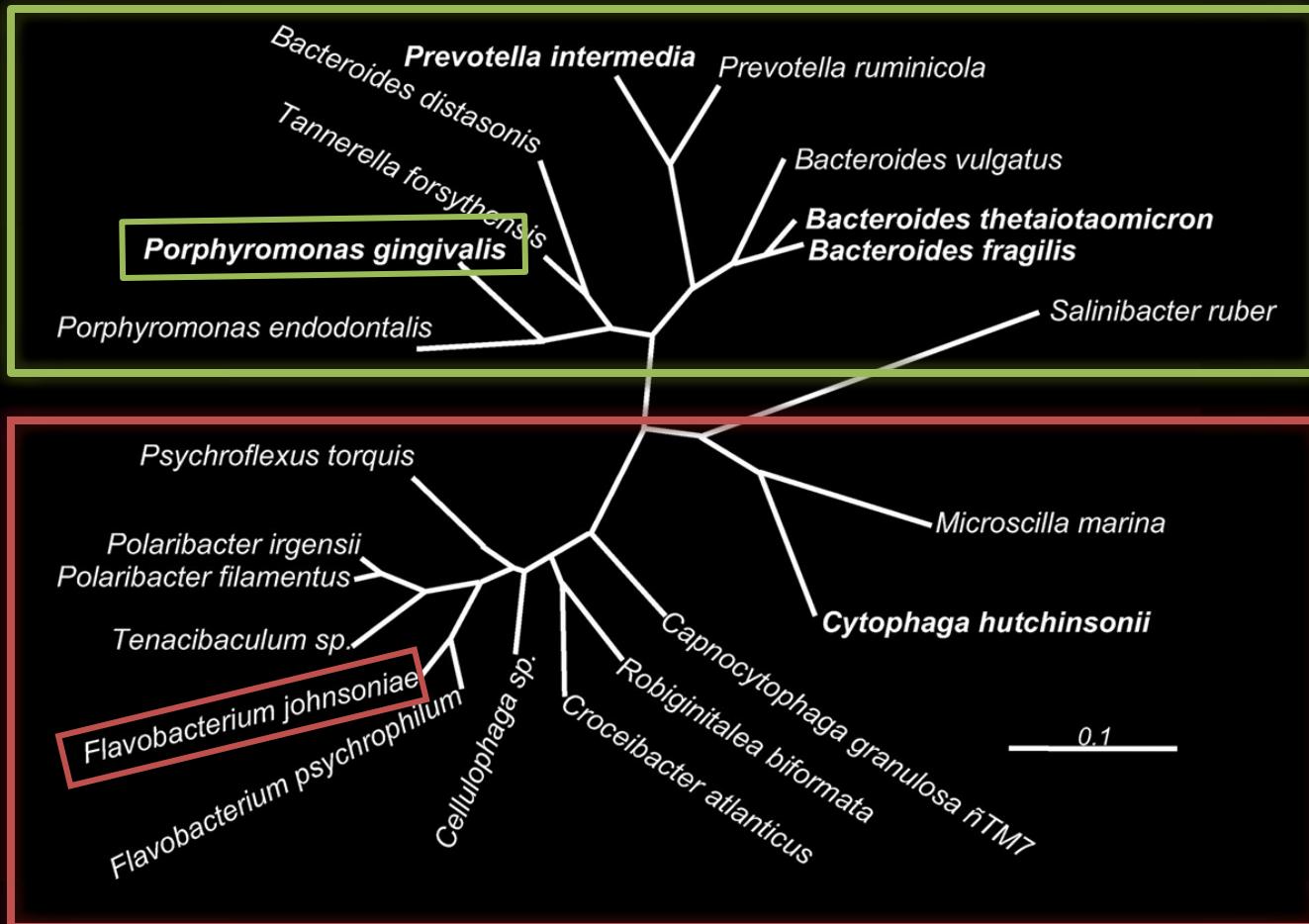
$\Delta sprB$ Cells + Beads + Anti-SprB



How Does SprB Reach the Cell Surface?

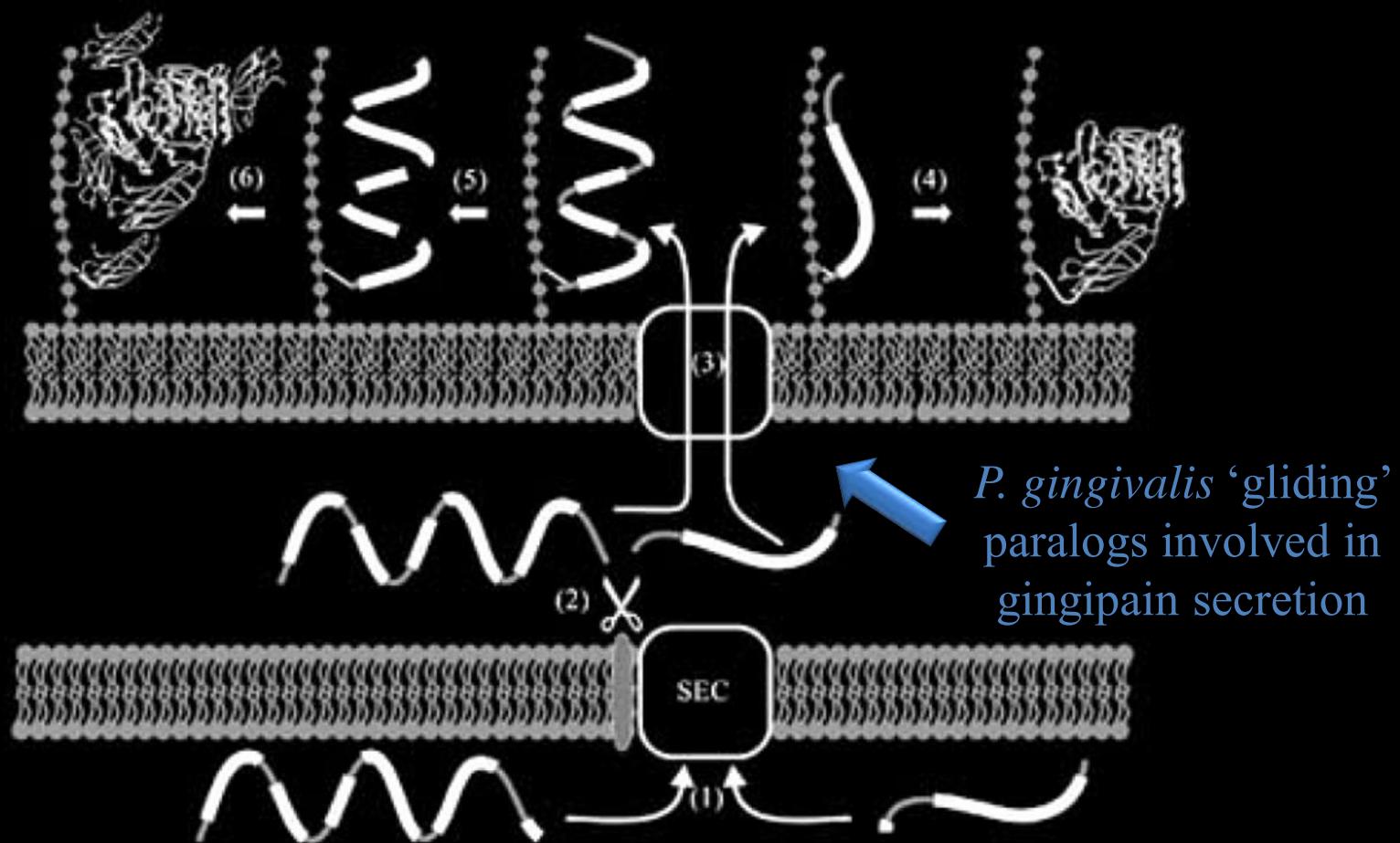
Bacteroidetes

Nonmotile but carry some 'gliding' genes

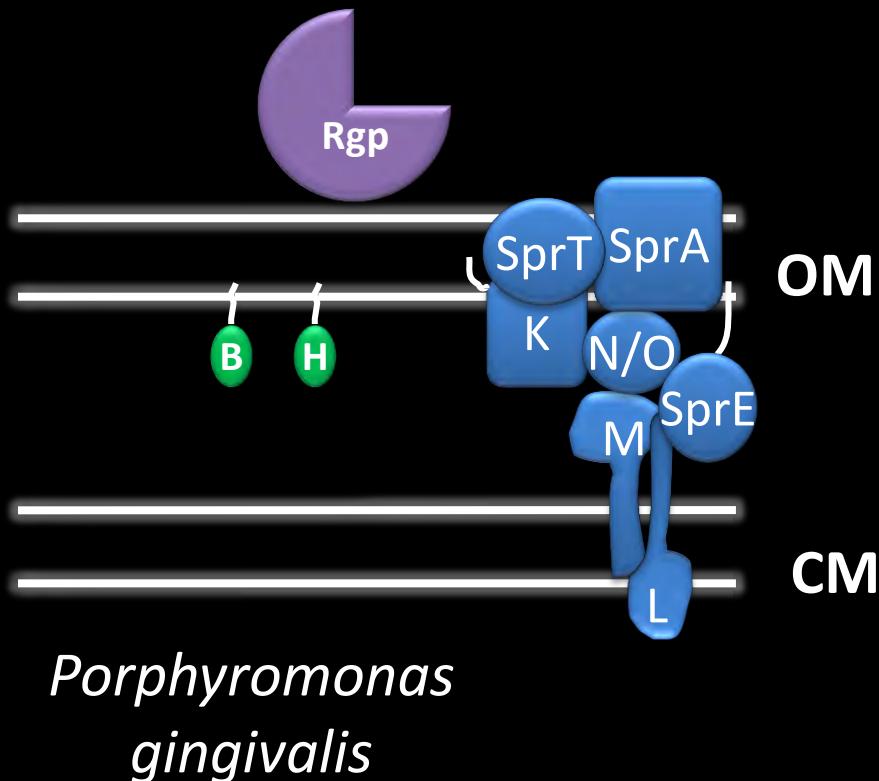
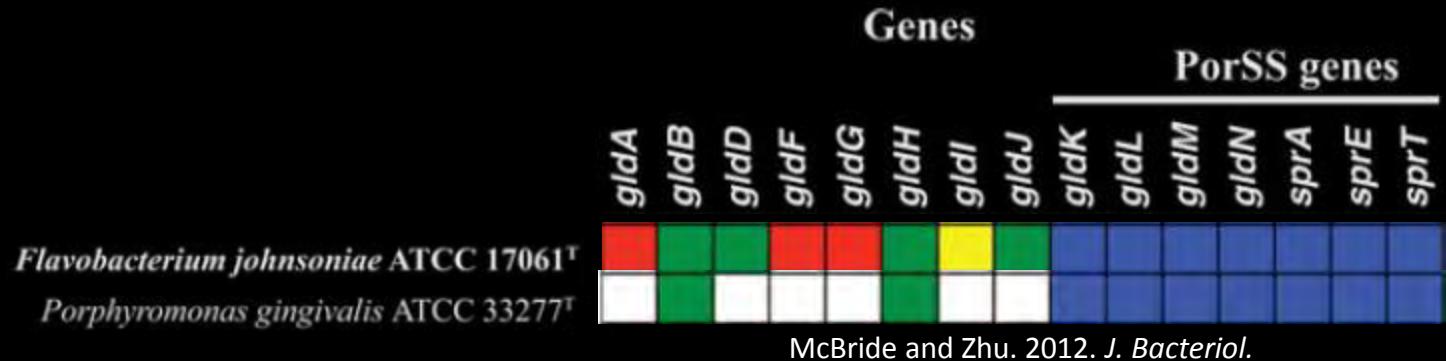


Motile and carry full complement of 'gliding' genes

Gingipain Secretion in *Porphyromonas gingivalis*



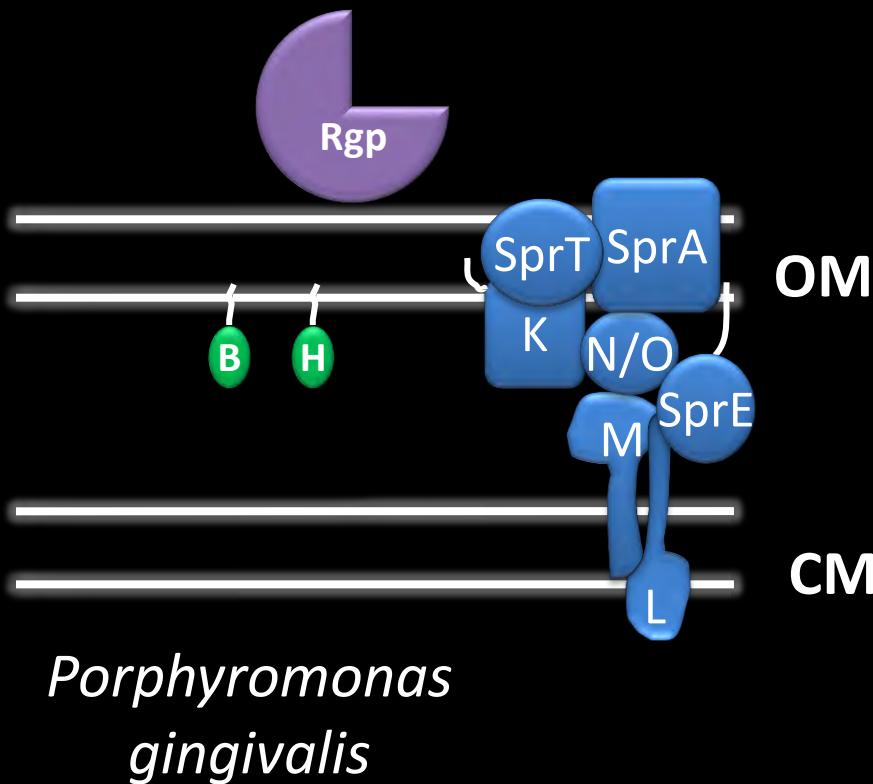
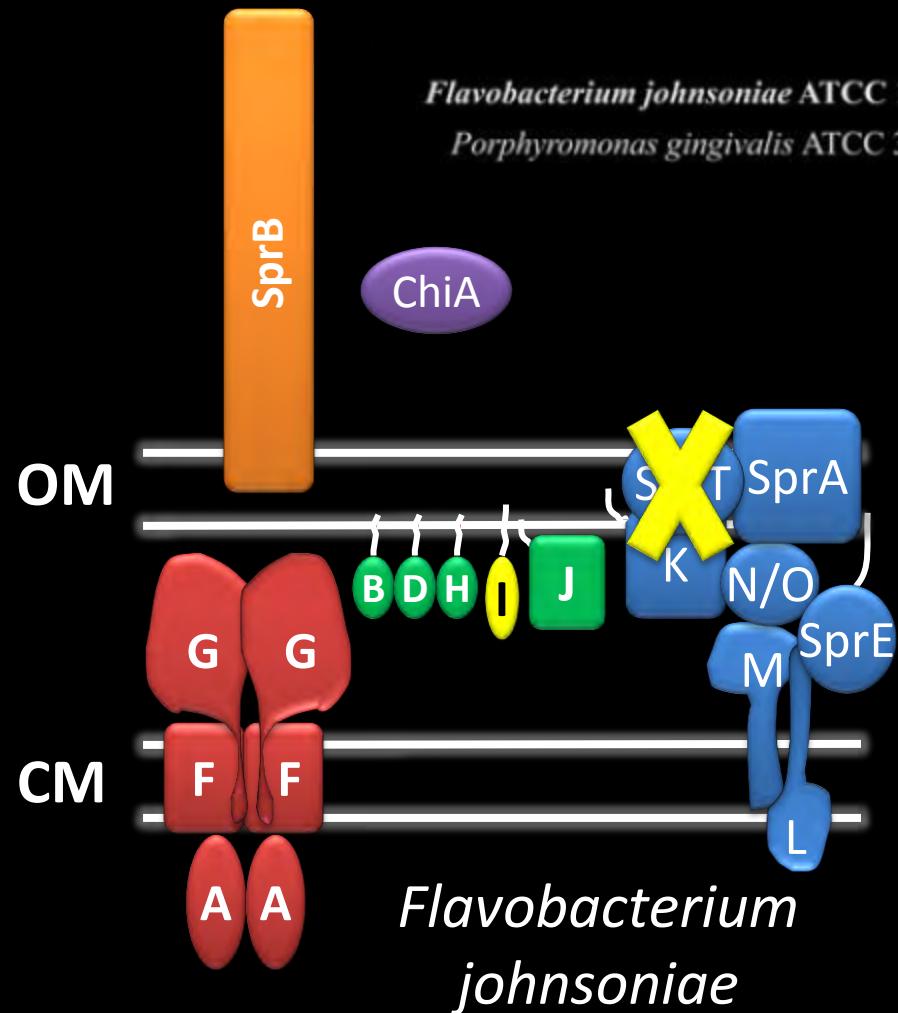
'Motility' Proteins



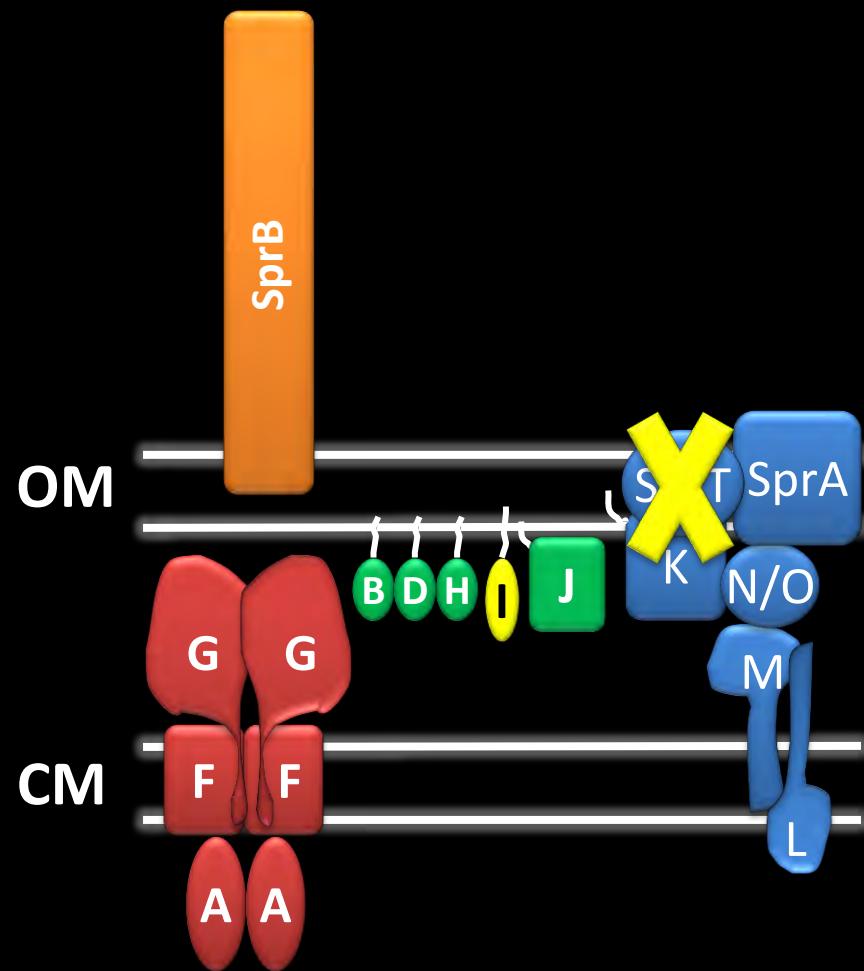
'Motility' Proteins

Genes	PorSS genes													
	<i>gldA</i>	<i>gldB</i>	<i>gldD</i>	<i>gldF</i>	<i>gldG</i>	<i>gldH</i>	<i>gldI</i>	<i>gldJ</i>	<i>gldK</i>	<i>gldL</i>	<i>gldM</i>	<i>gldN</i>	<i>sprA</i>	<i>sprE</i>
<i>Flavobacterium johnsoniae</i> ATCC 17061 ^T	Red	Green	Red	Red	Green	Yellow	Green	Blue						
<i>Porphyromonas gingivalis</i> ATCC 33277 ^T	White													

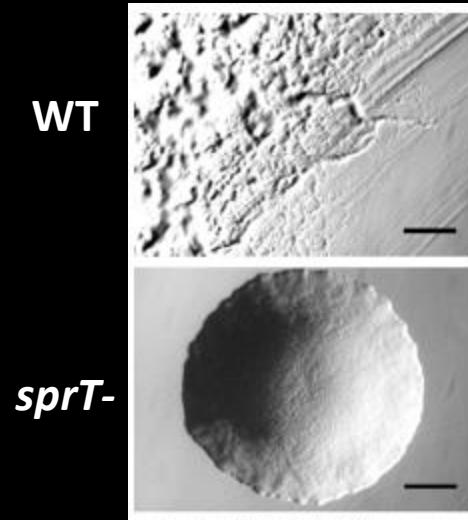
McBride and Zhu. 2012. *J. Bacteriol.*



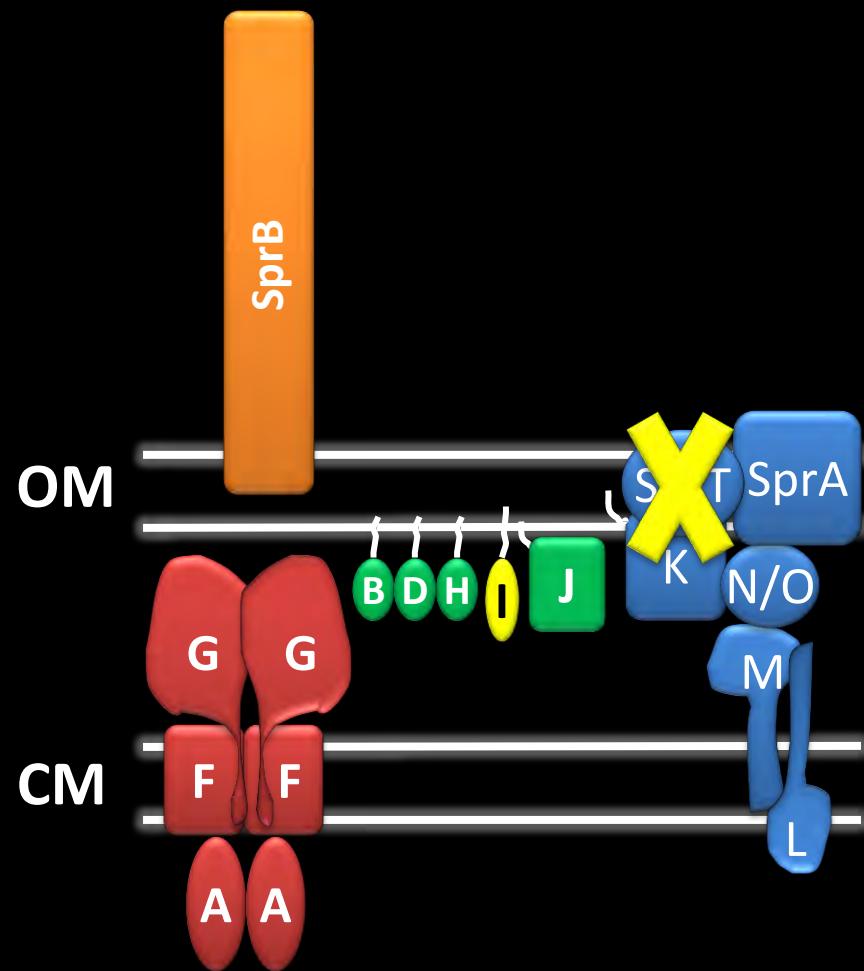
SprT is Necessary for Motility and Chitinase Activity



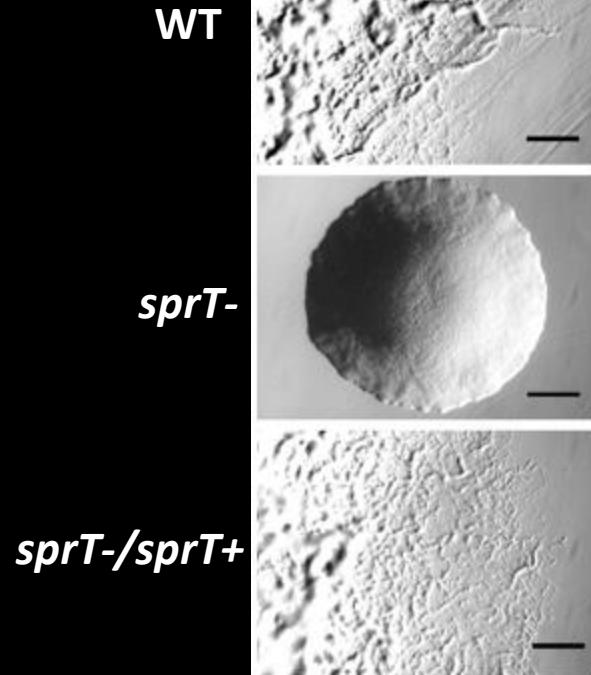
Colony Spreading



SprT is Necessary for Motility and Chitinase Activity

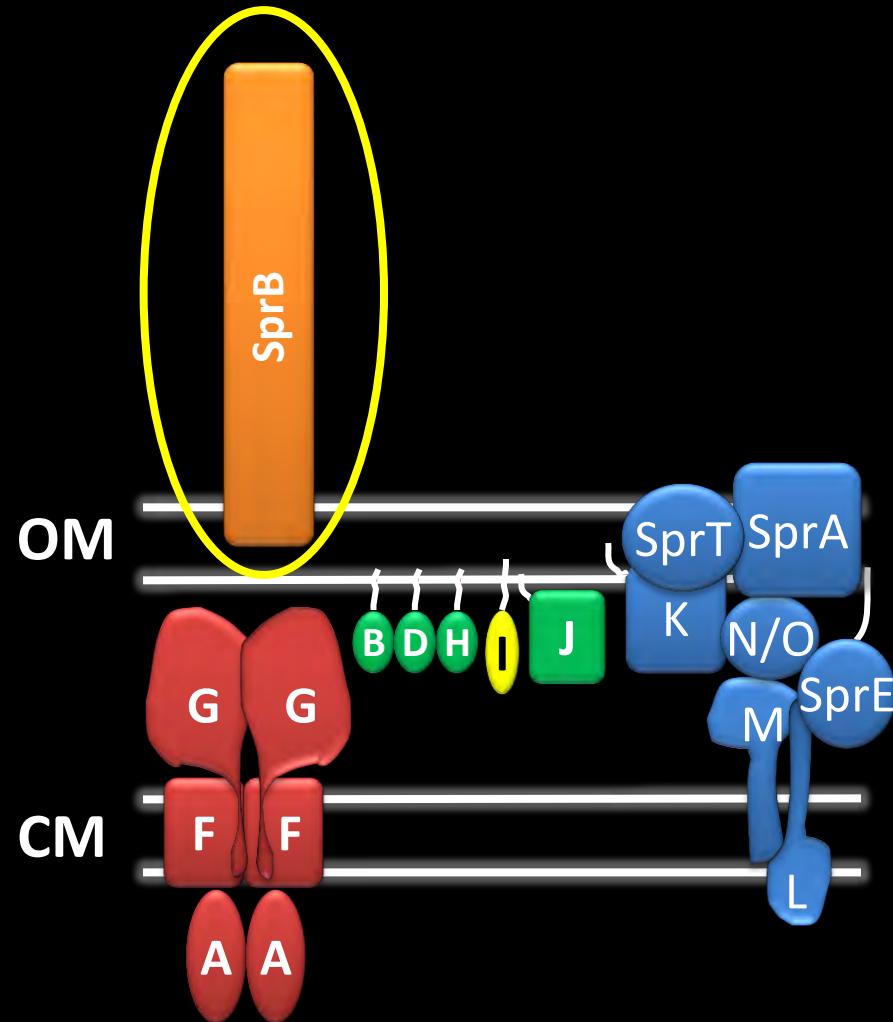
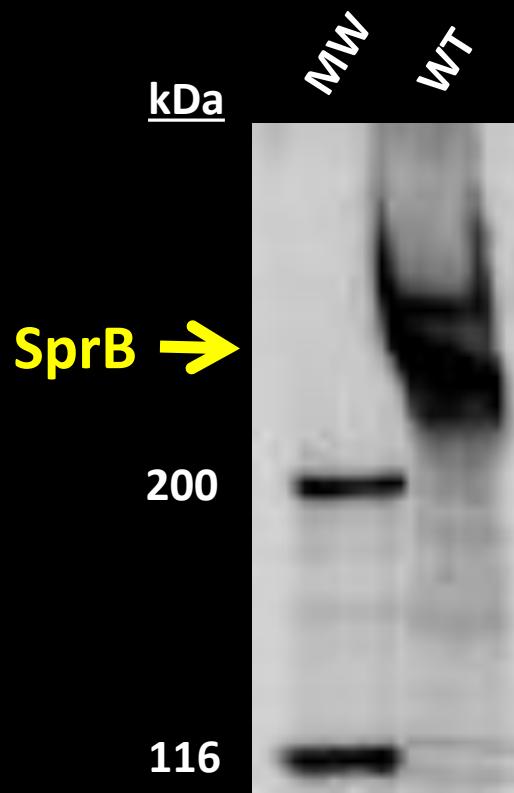


Colony Spreading



Is SprT Required for SprB Localization to the Cell Surface?

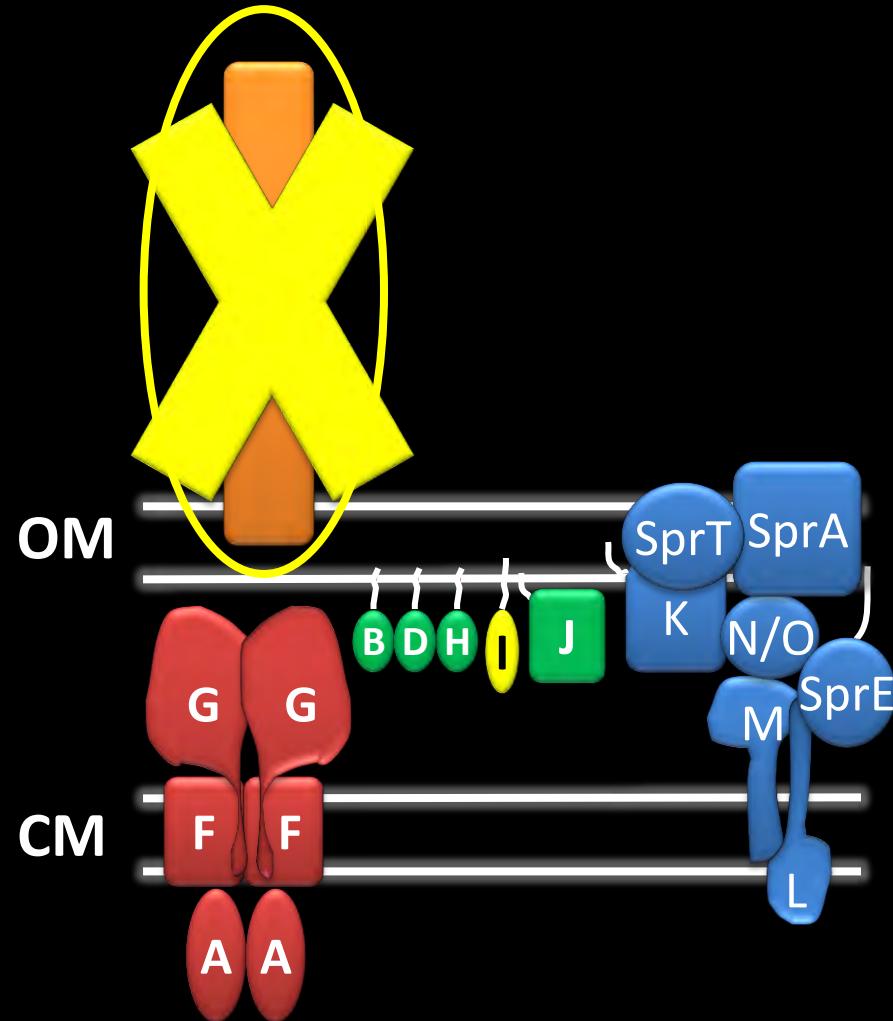
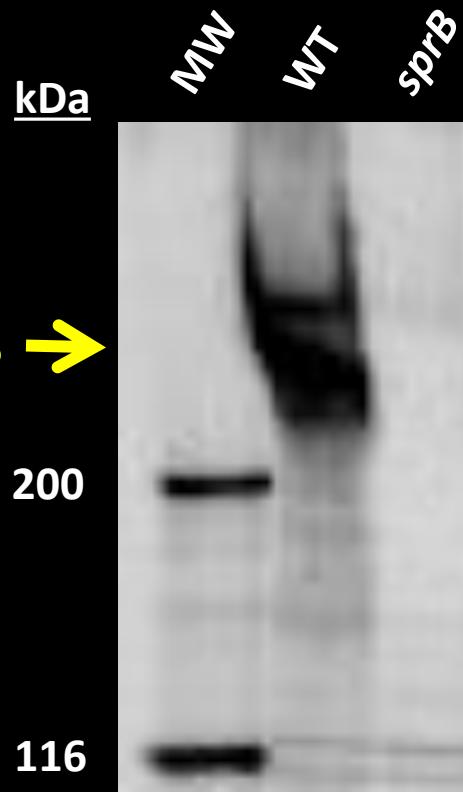
SprB in Whole Cells



Sato, Naito, Yukitake, Hirakawa, Shoji, McBride, Rhodes and Nakayama. 2010. *Proc Natl Acad Sci.*

Is SprT Required for SprB Localization to the Cell Surface?

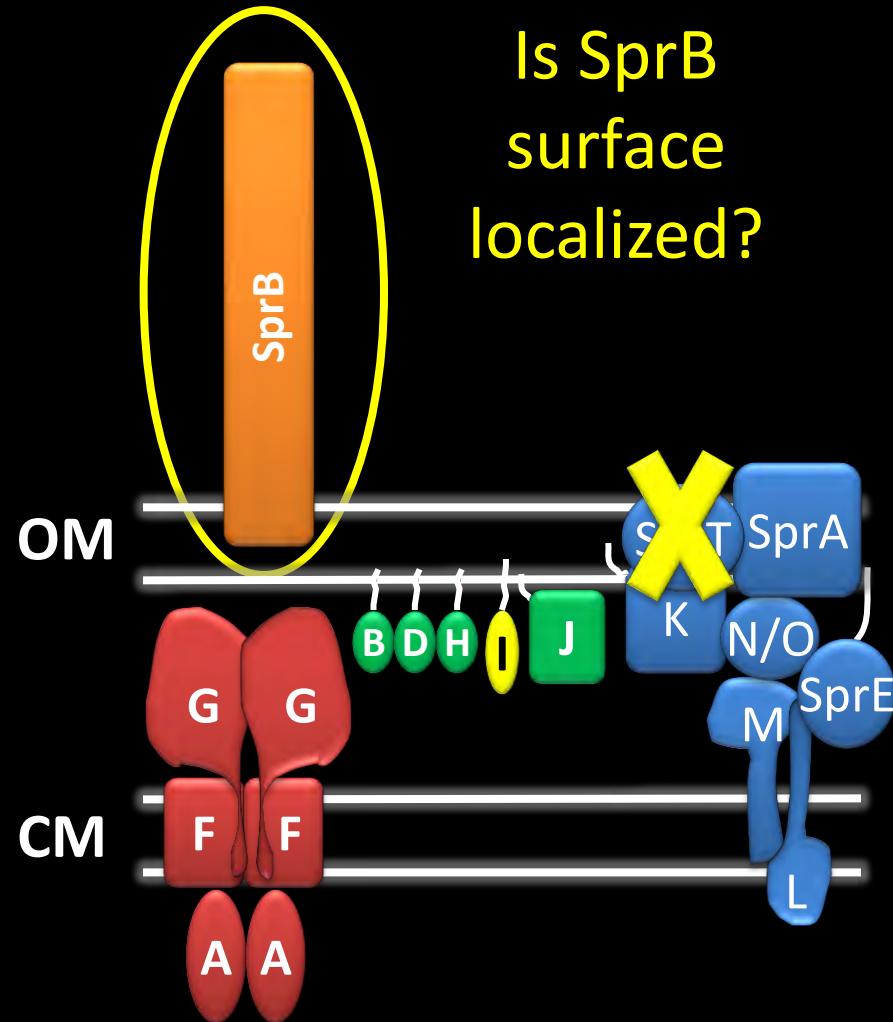
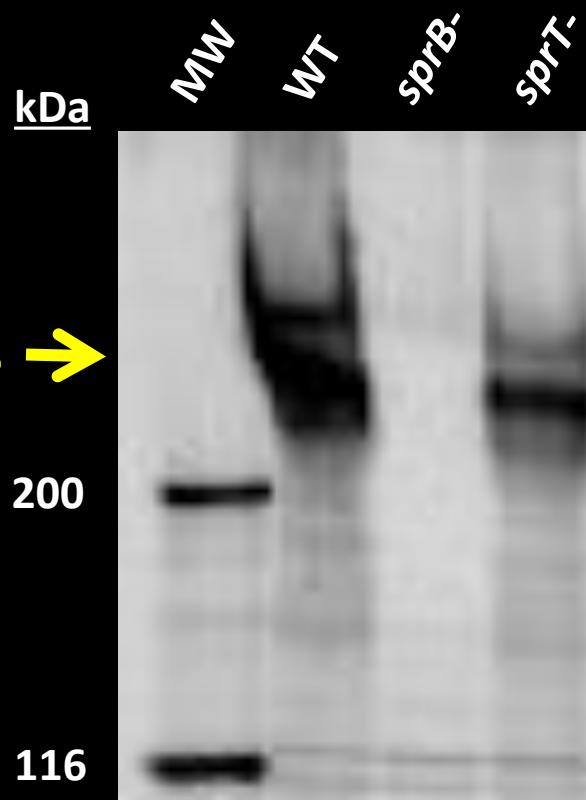
SprB in Whole Cells



Sato, Naito, Yukitake, Hirakawa, Shoji, McBride, Rhodes and Nakayama. 2010. *Proc Natl Acad Sci.*

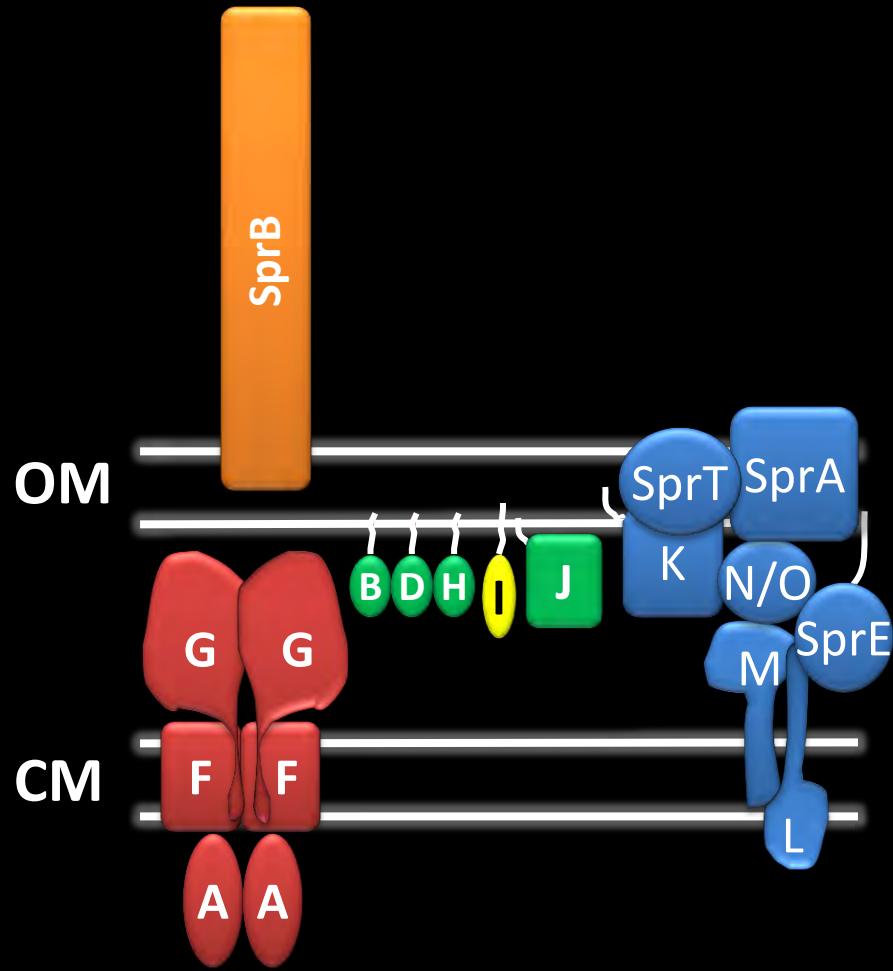
Is SprT Required for SprB Localization to the Cell Surface?

SprB in Whole Cells



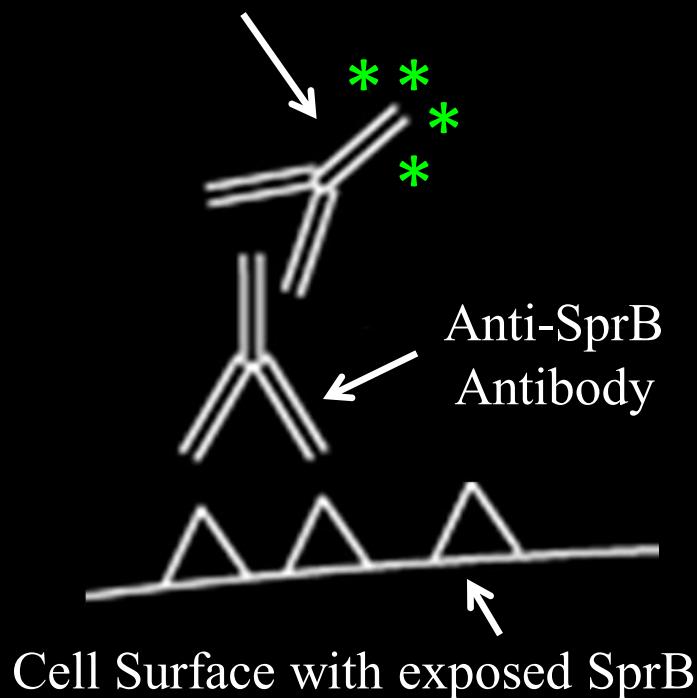
Sato, Naito, Yukitake, Hirakawa, Shoji, McBride, Rhodes and Nakayama. 2010. *Proc Natl Acad Sci.*

Is SprT Required for SprB Localization to the Cell Surface?

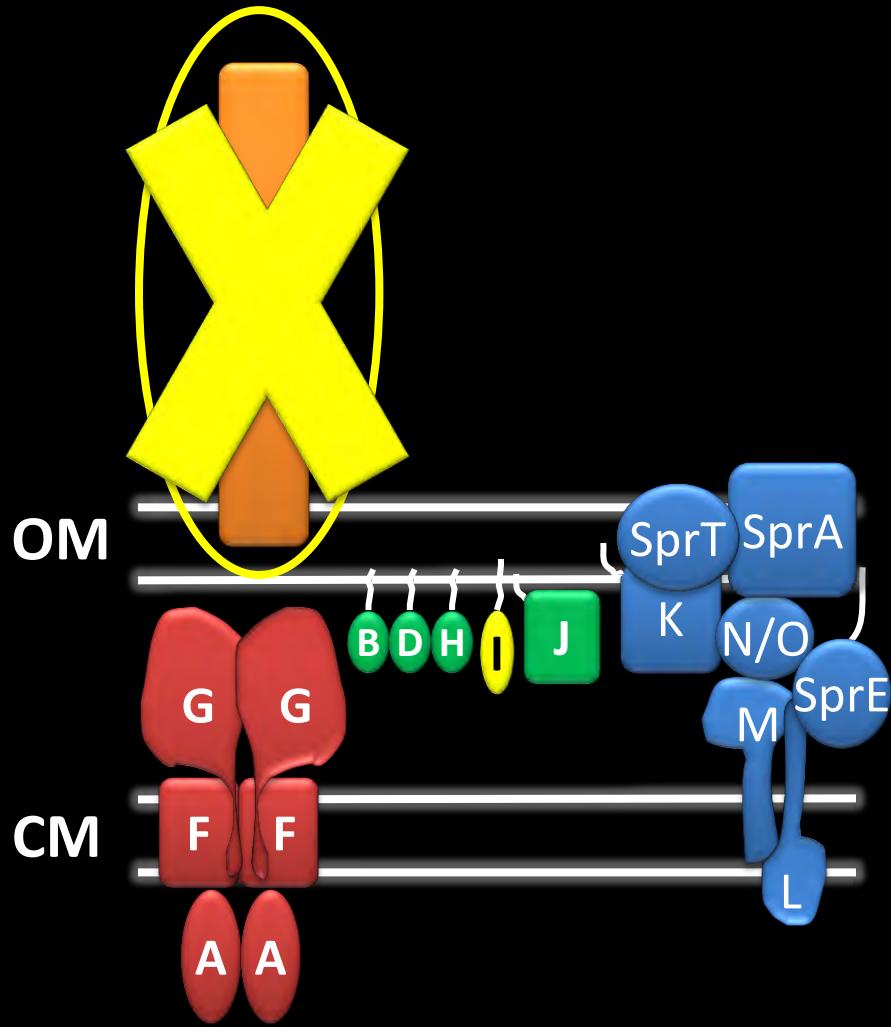


Detecting Surface Localized SprB

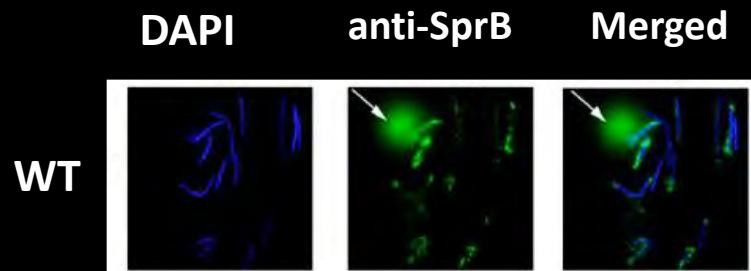
Fluorescently Labeled
Secondary Antibody



SprT Required for SprB Localization

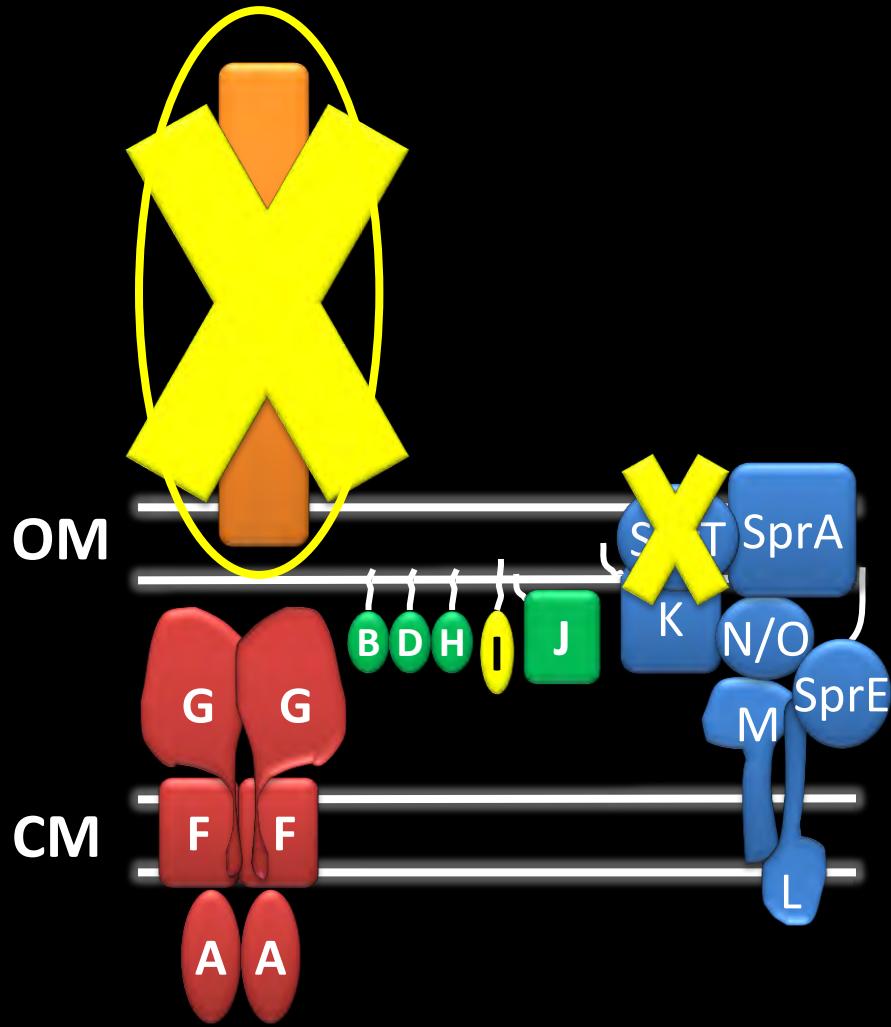


Surface Localized SprB

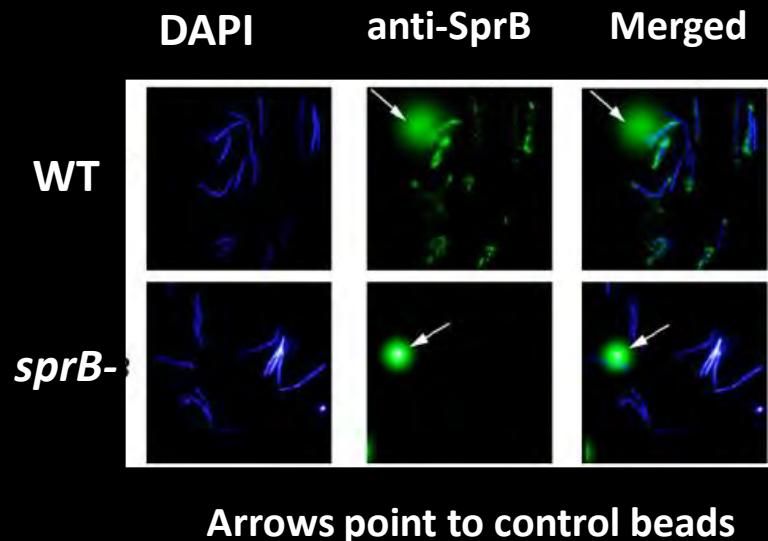


Arrows point to control beads

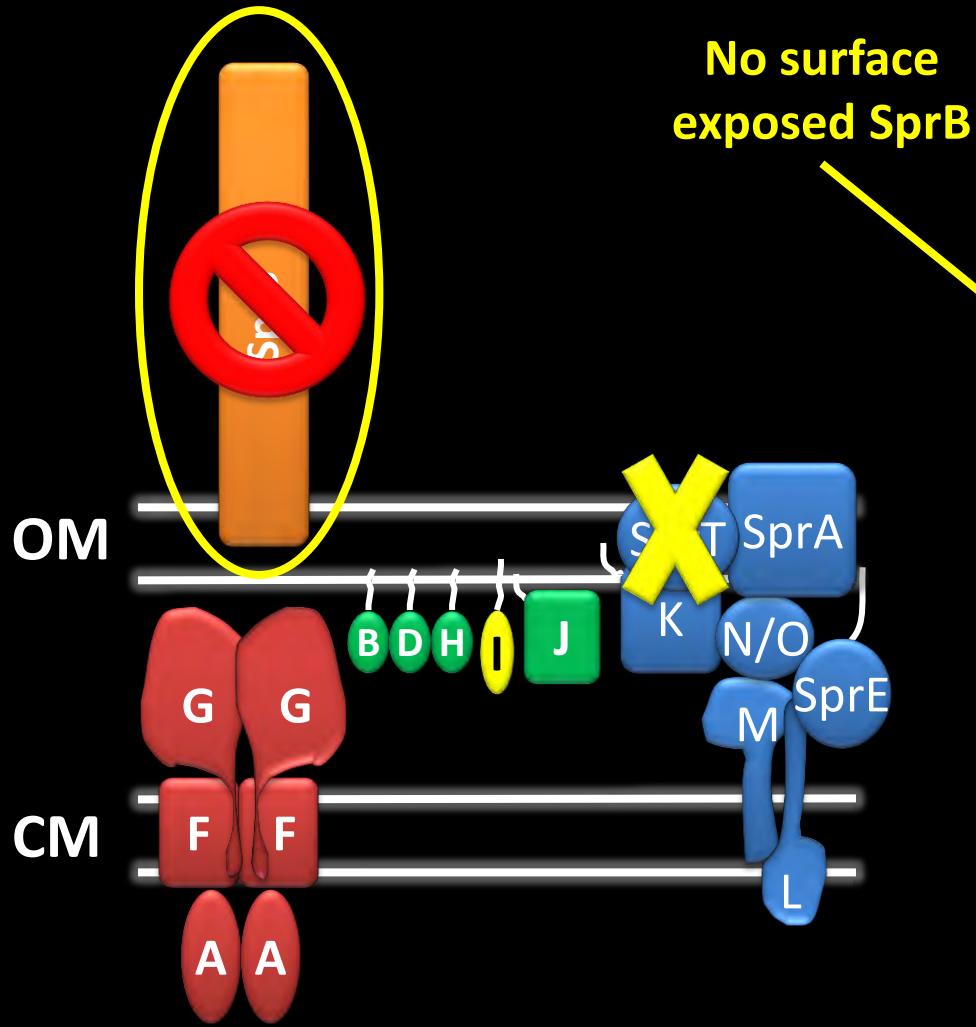
SprT Required for SprB Localization



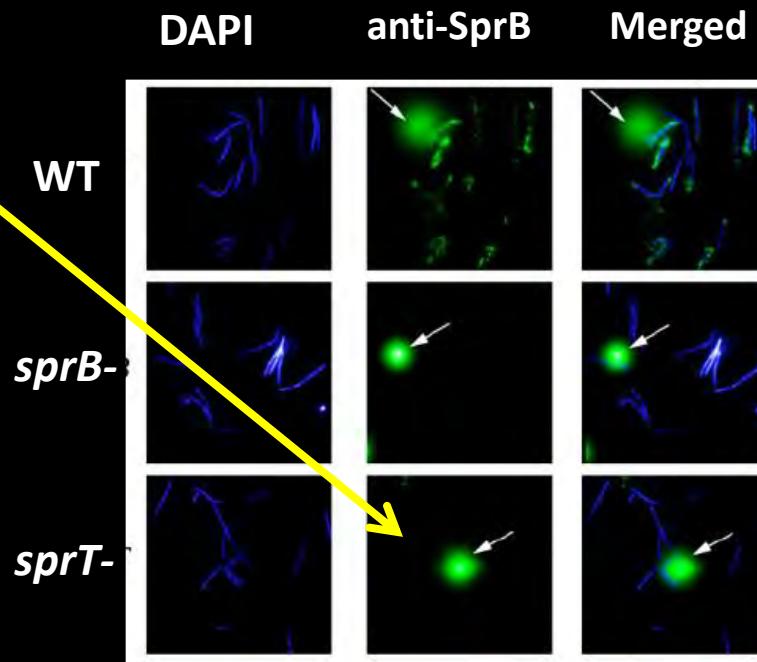
Surface Localized SprB



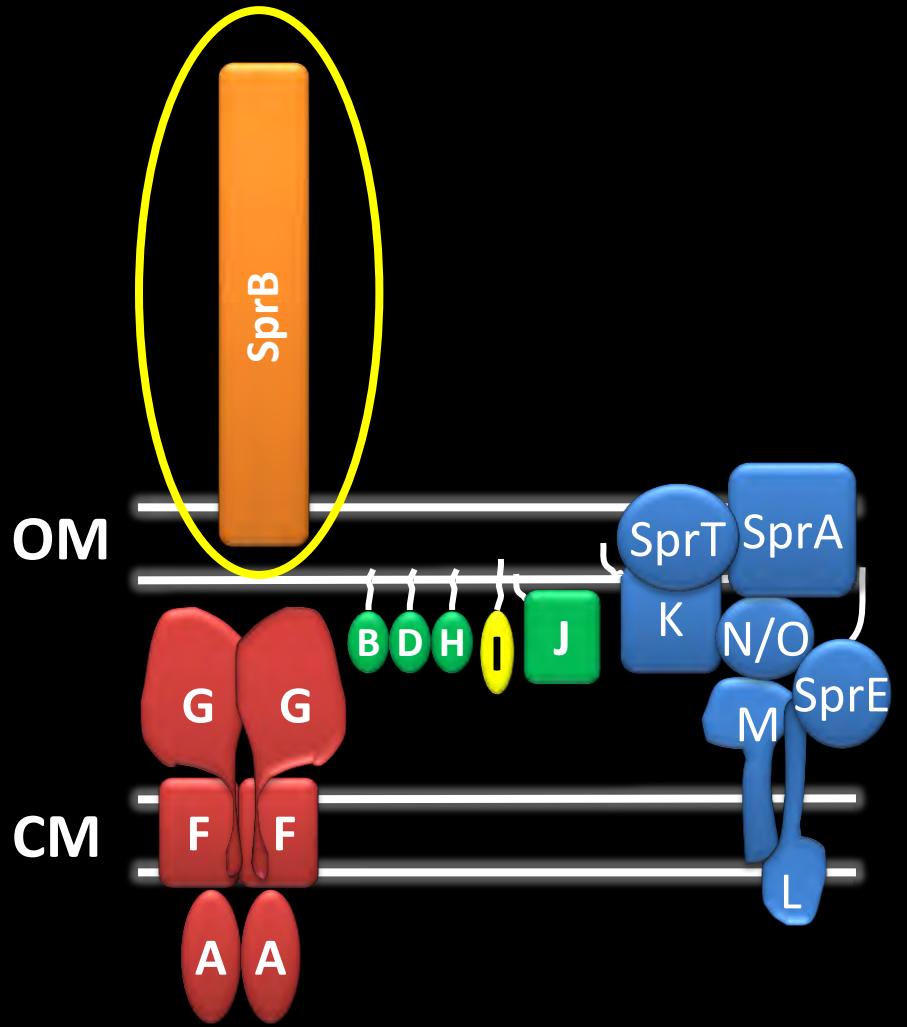
SprT Required for SprB Localization



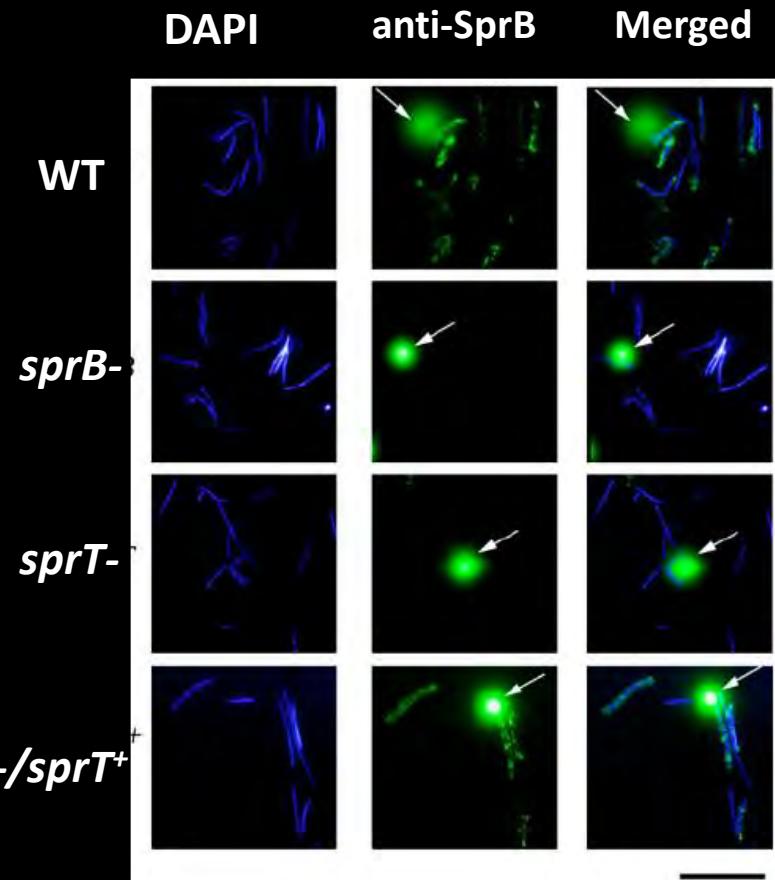
Surface Localized SprB



SprT Required for SprB Localization

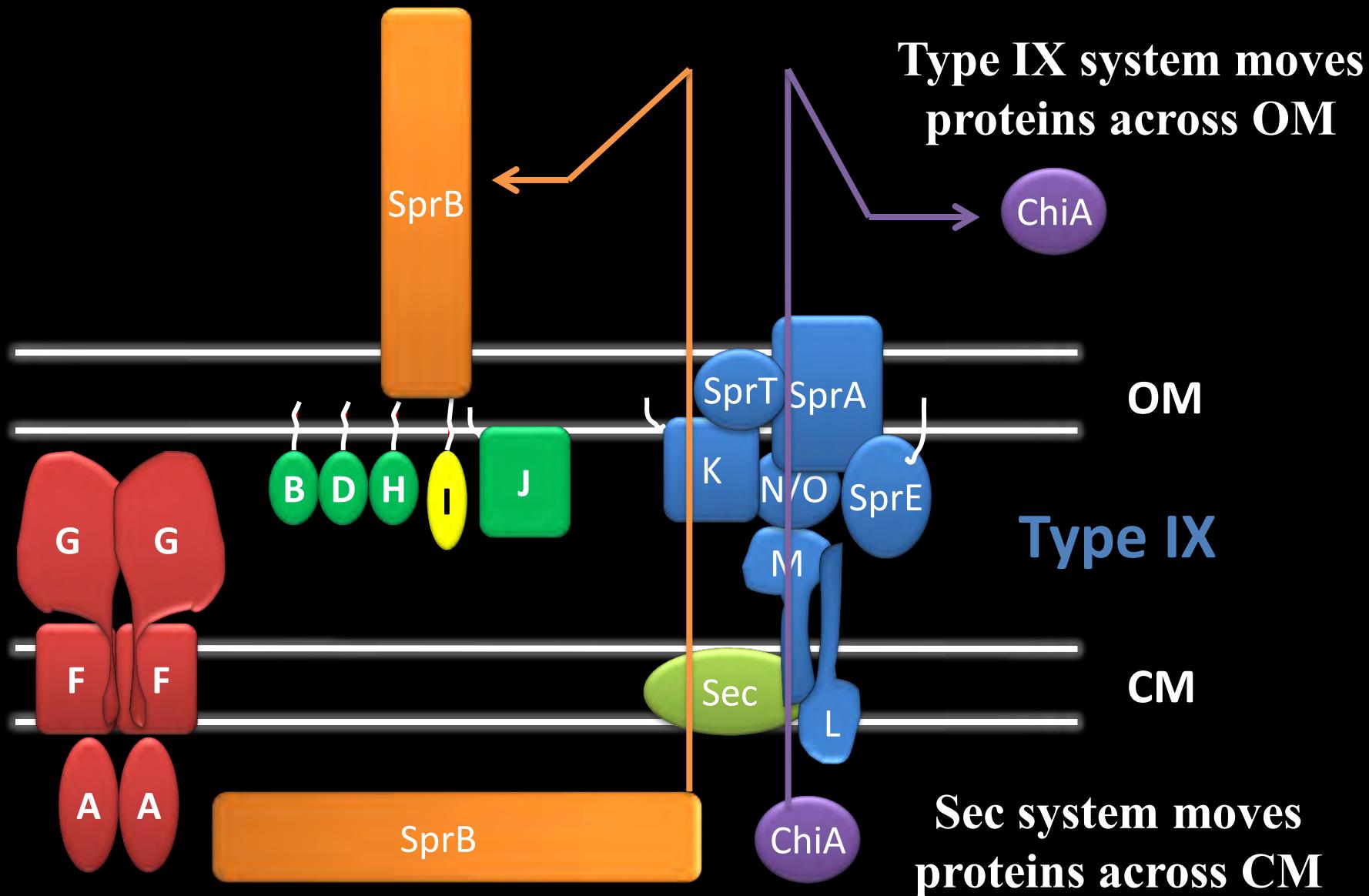


Surface Localized SprB



Sato, Naito, Yukitake, Hirakawa, Shoji, McBride, Rhodes and Nakayama. 2010. *Proc Natl Acad Sci.*

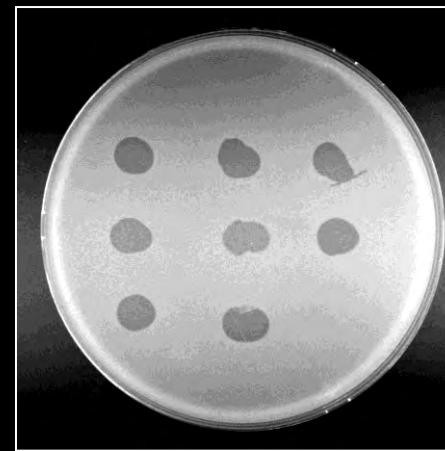
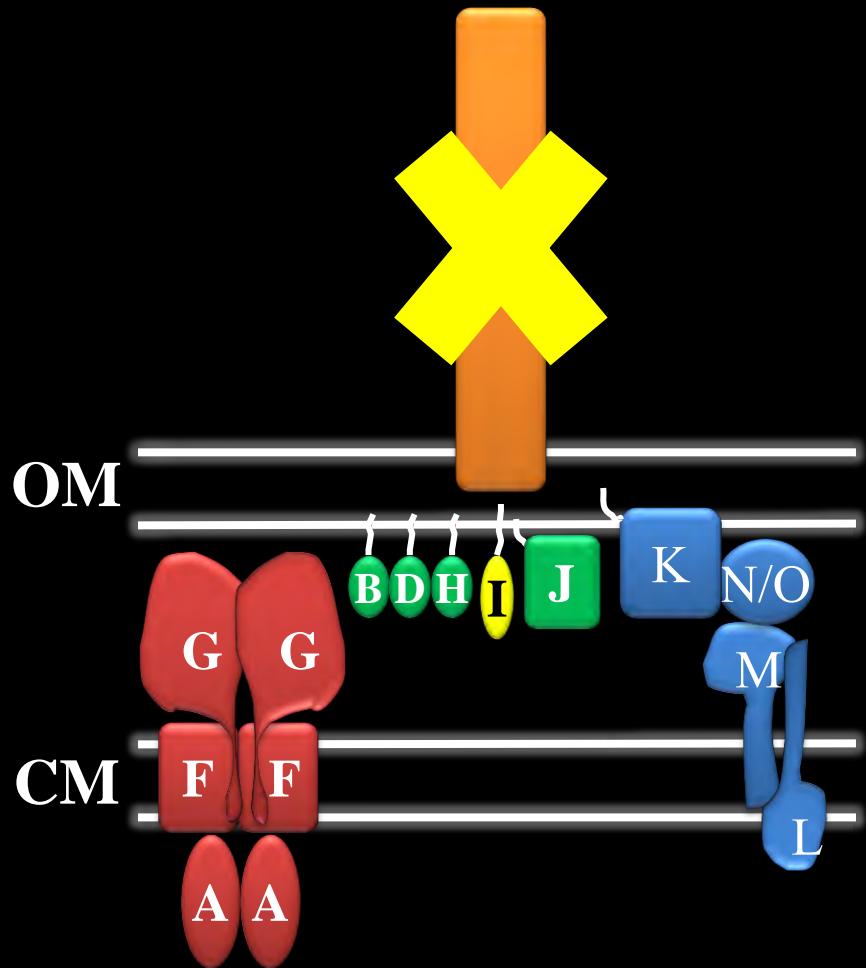
Secretion in *F. johnsoniae*



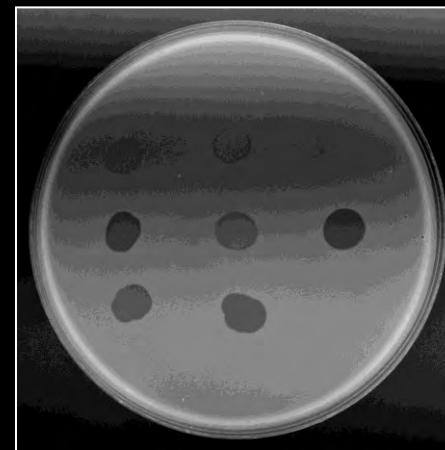
Are there other surface exposed
proteins involved in motility and/or
adhesion?

How do we investigate this?

Phage Resistance of *sprB* Mutant



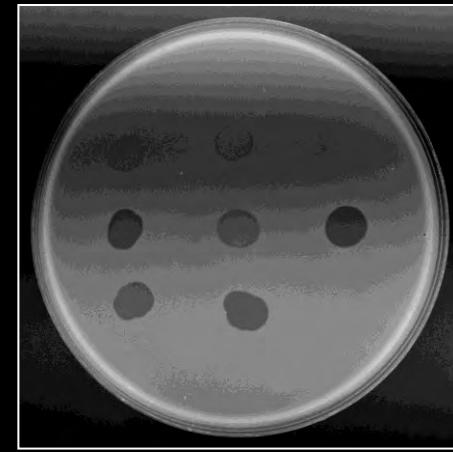
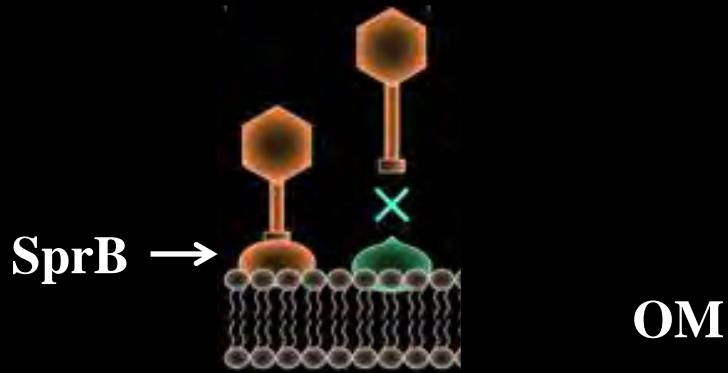
WT



$\Delta sprB$

Nelson, Bollampalli and McBride. 2008. *J Bacteriol.*

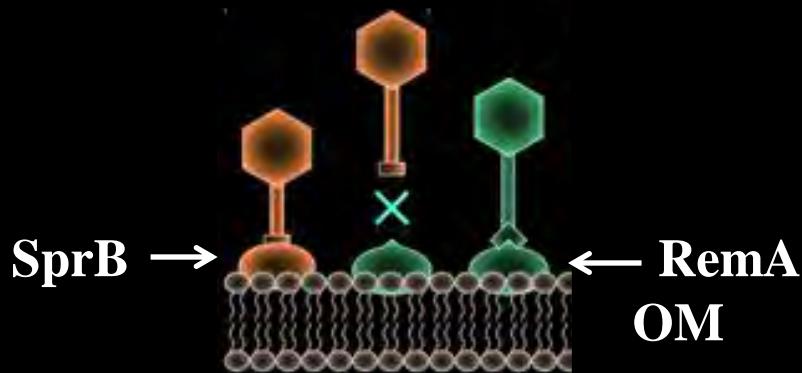
Random Mutagenesis of *sprB* Mutant



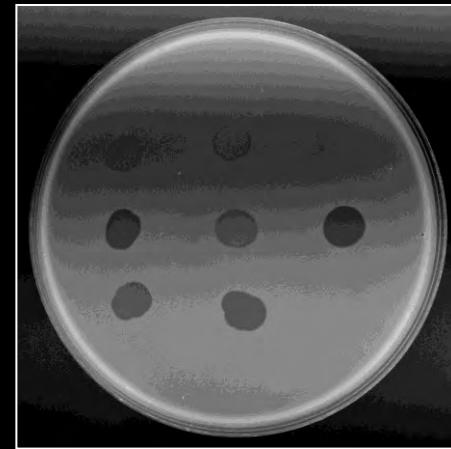
$\Delta sprB$

<http://www.nature.com/nrmicro/journal/v8/n5/>

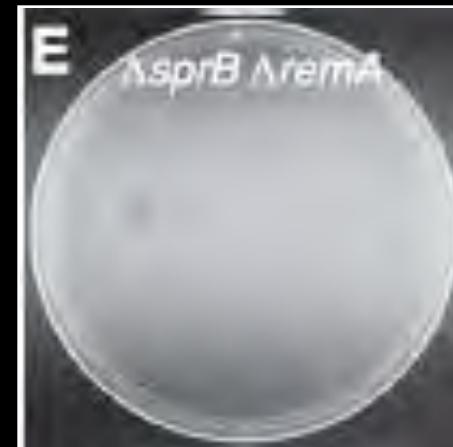
Random Mutagenesis of *sprB* Mutant



<http://www.nature.com/nrmicro/journal/v8/n5/>



$\Delta sprB$



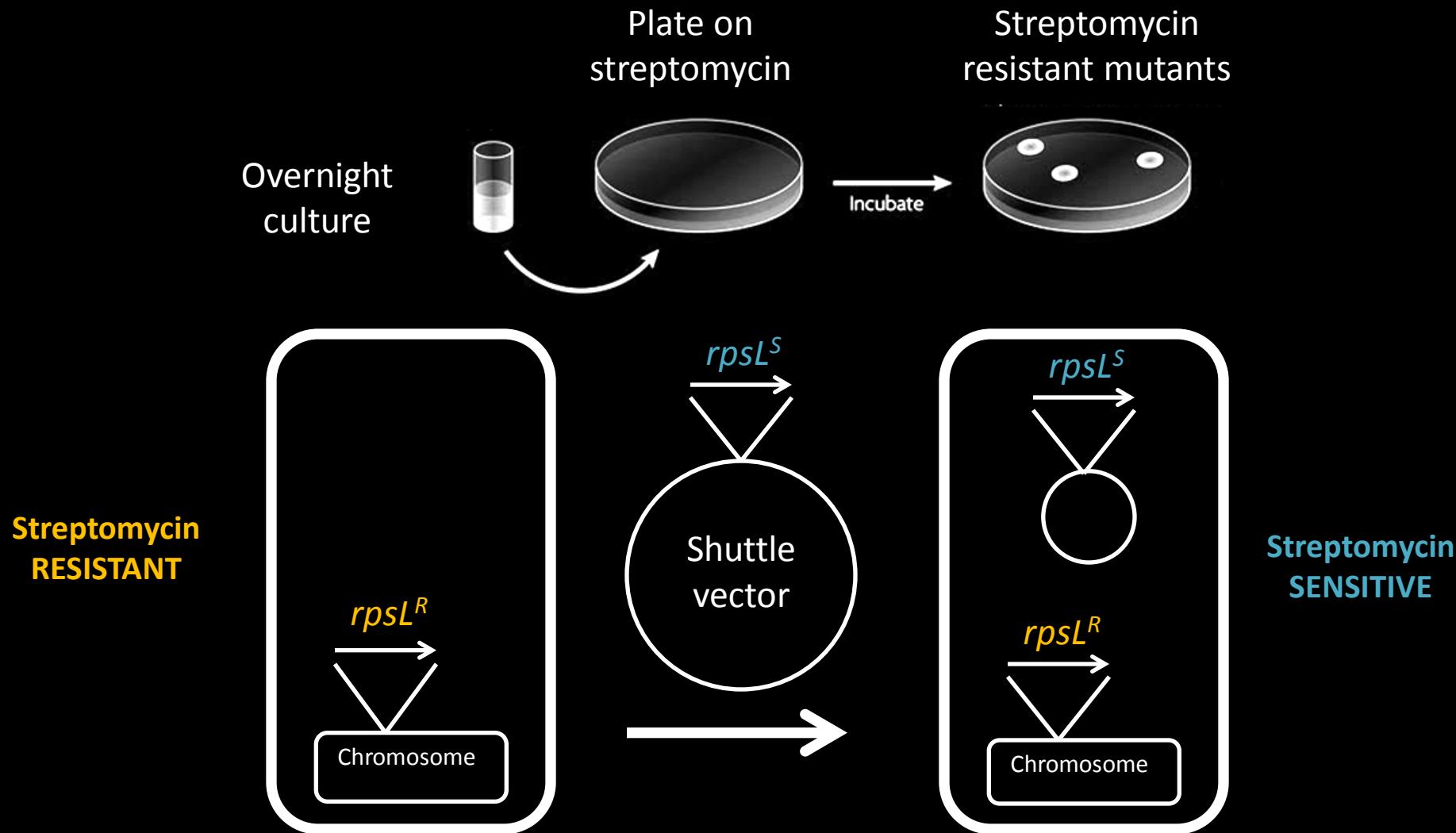
$\Delta sprB \Delta remA$

RemA is Surface Exposed

Can we follow the movement of
RemA on the surface? How?

“Tag” RemA with a myc epitope
N-EQKLISEEDL-C

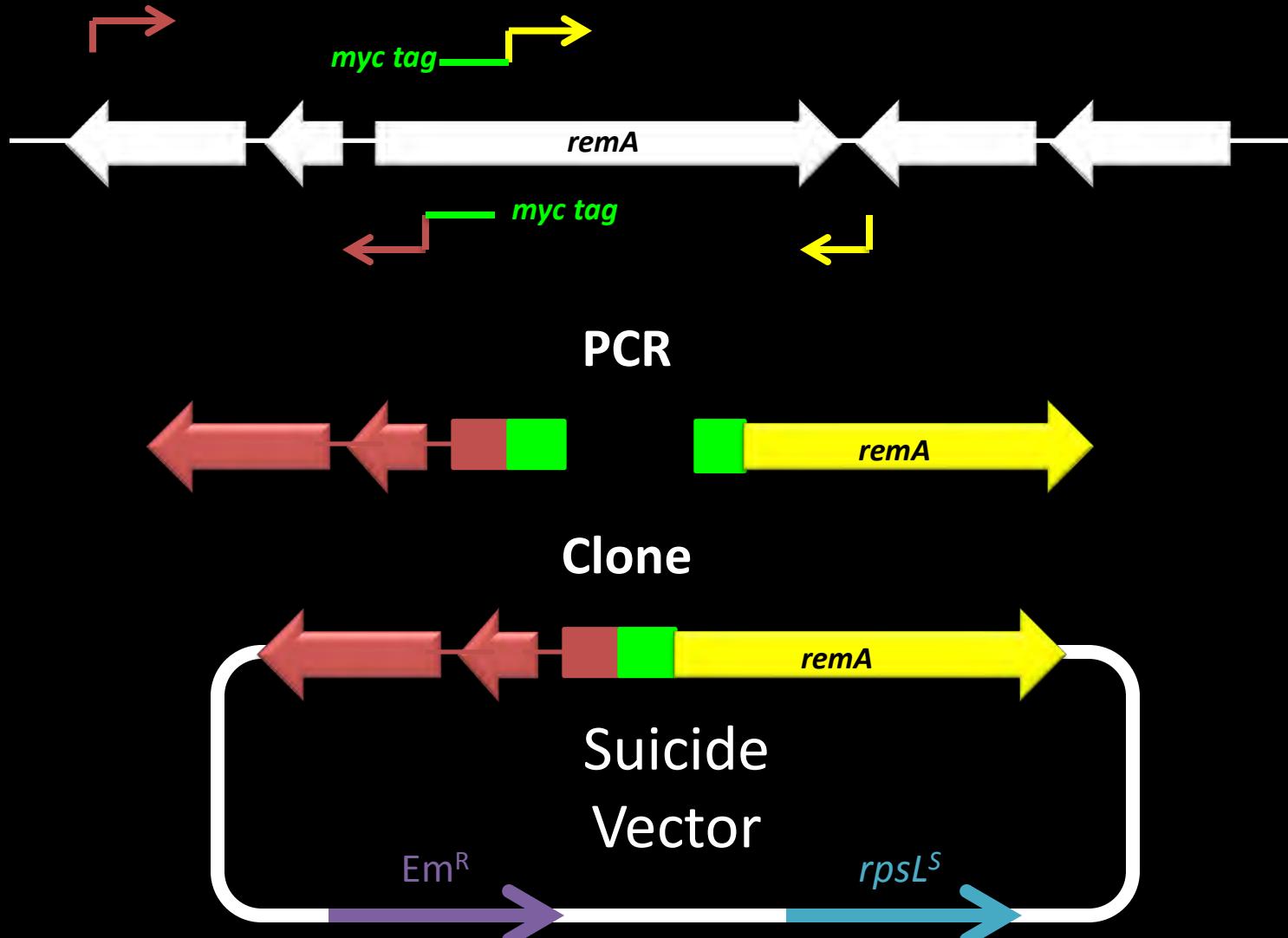
Gene Deletion System



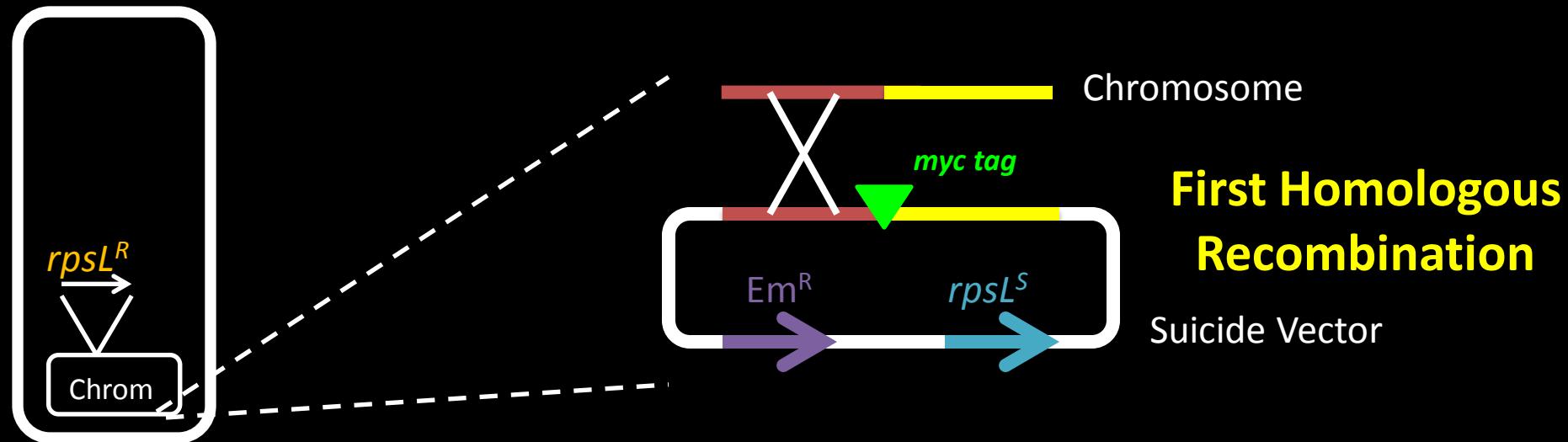
Myc “Tag” Insertion in RemA



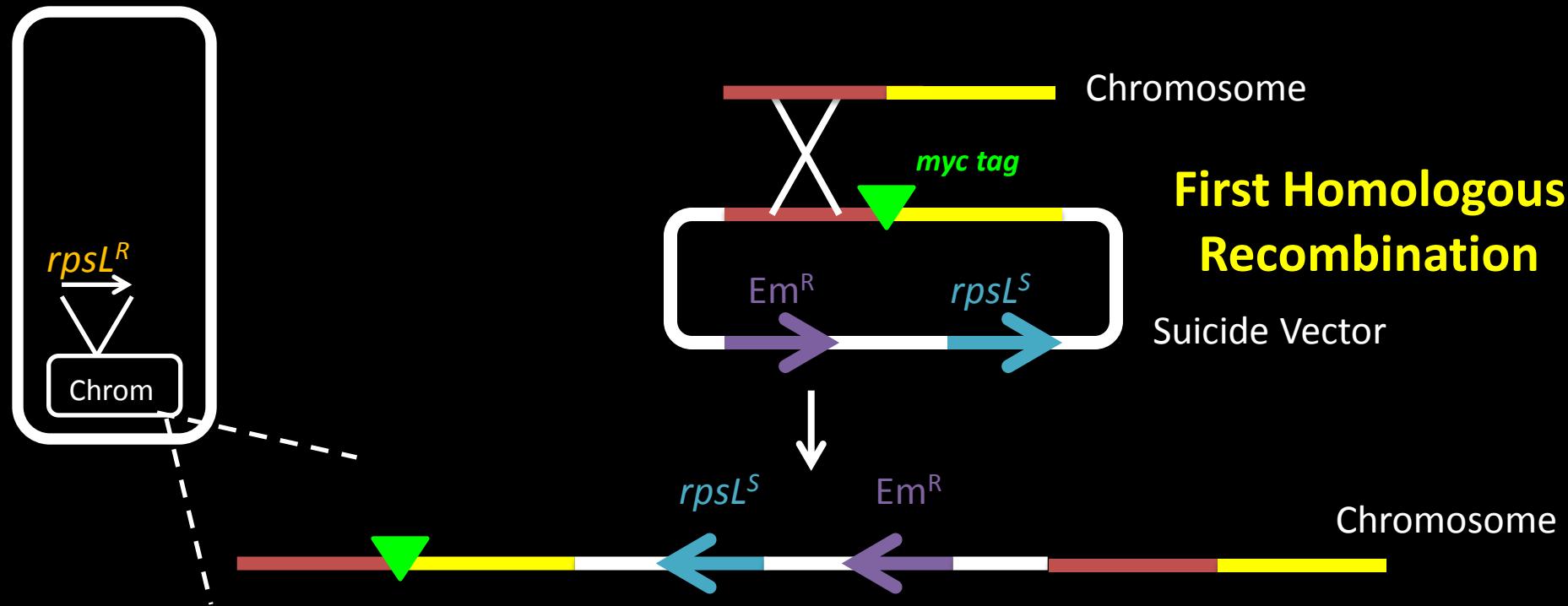
Myc “Tag” Insertion in RemA



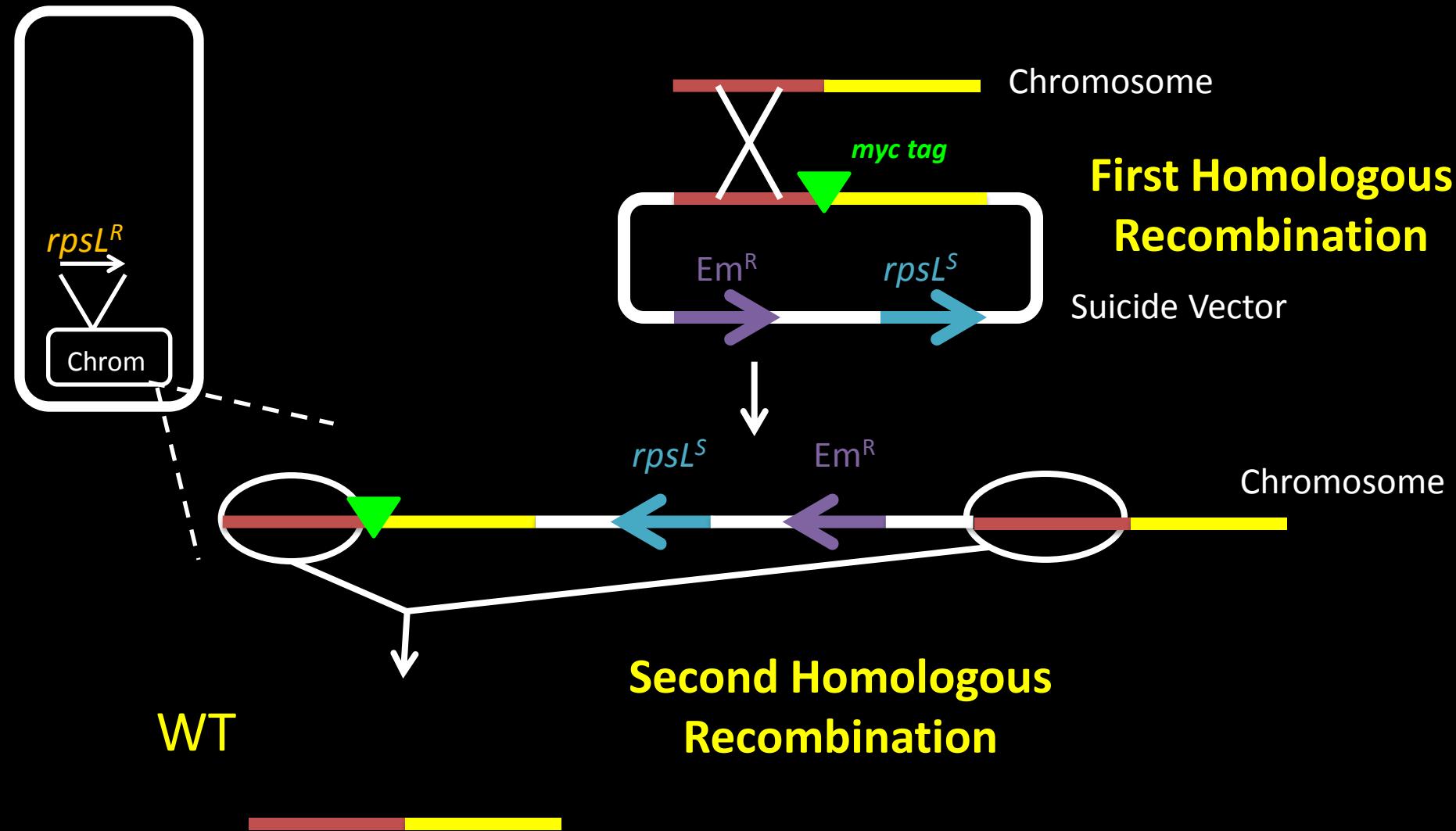
Myc “Tag” Insertion in RemA



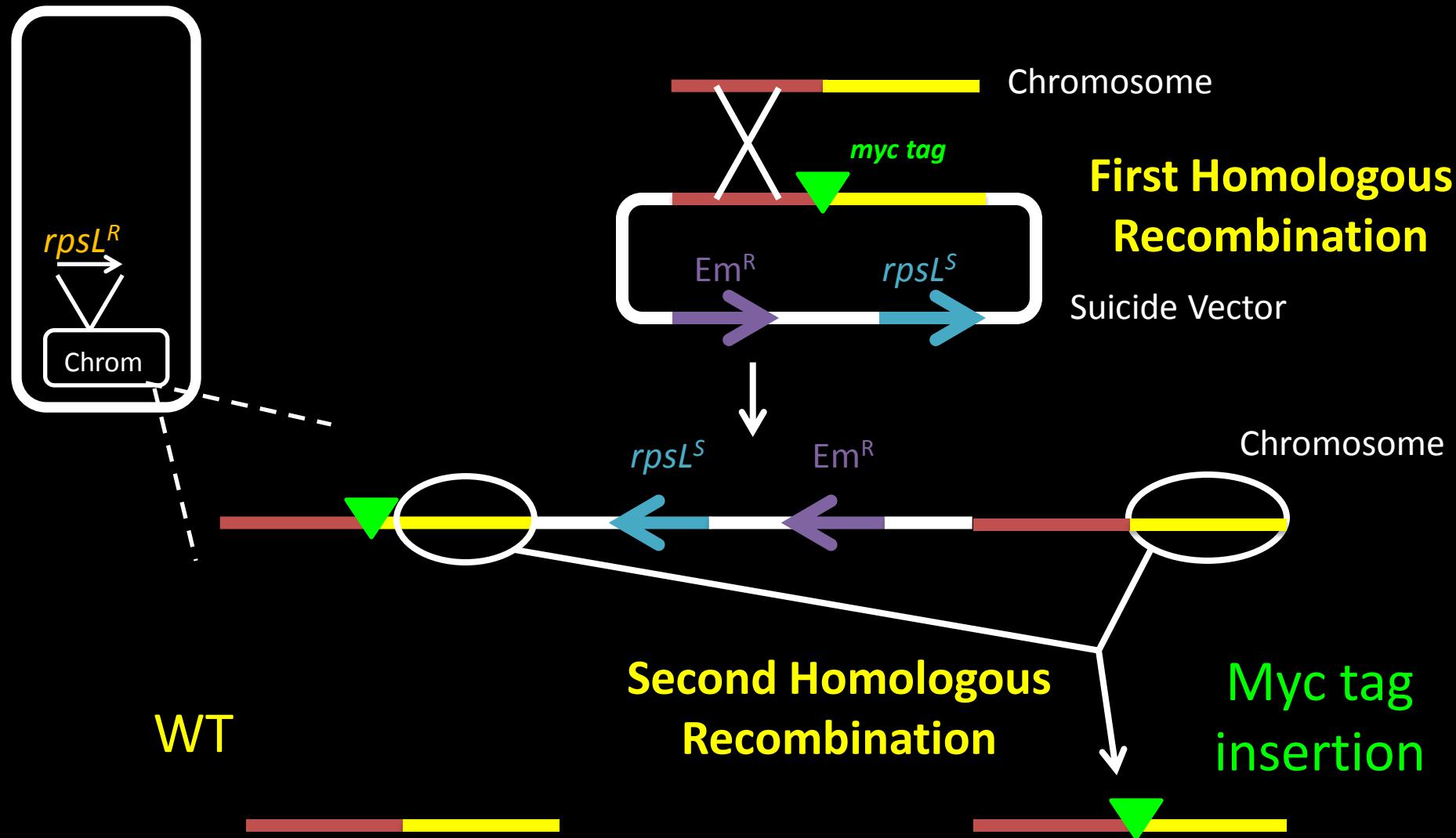
Myc “Tag” Insertion in RemA



Myc “Tag” Insertion in RemA



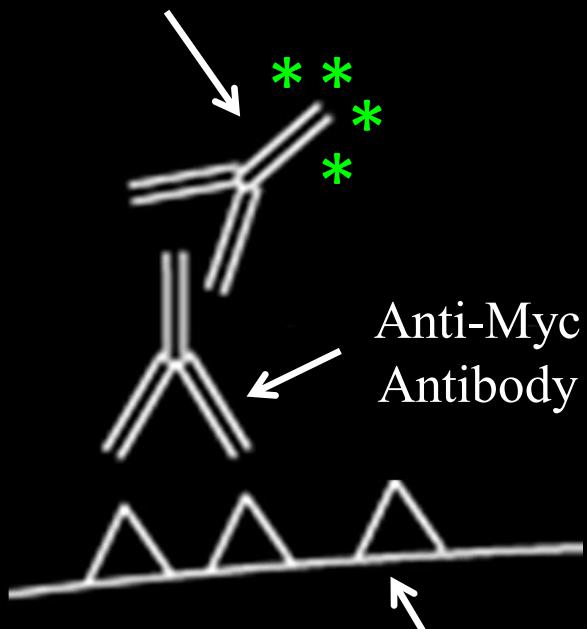
Myc “Tag” Insertion in RemA



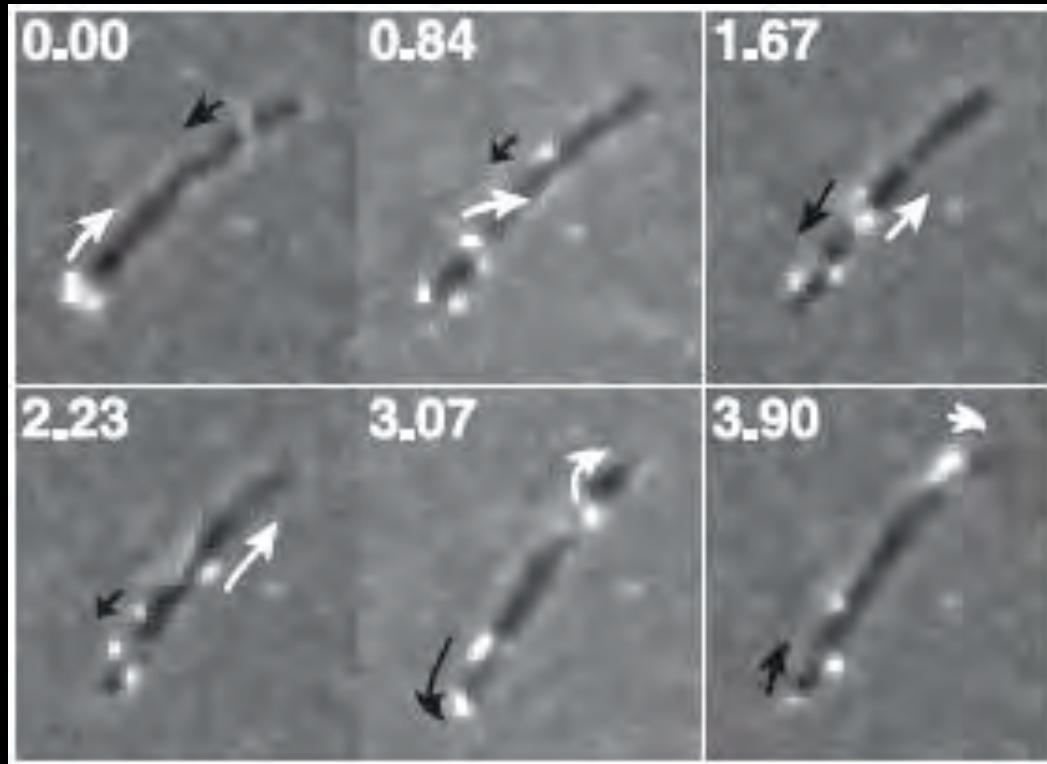
Movement of RemA on Cell Surface

Detecting Surface Localized RemA-Myc

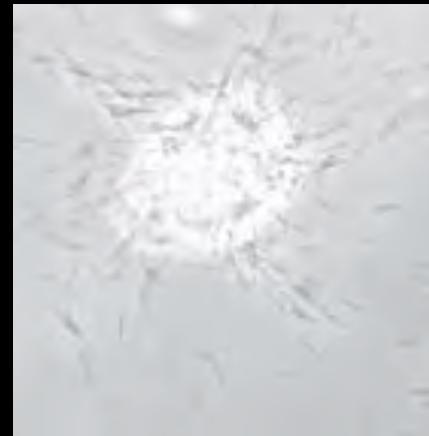
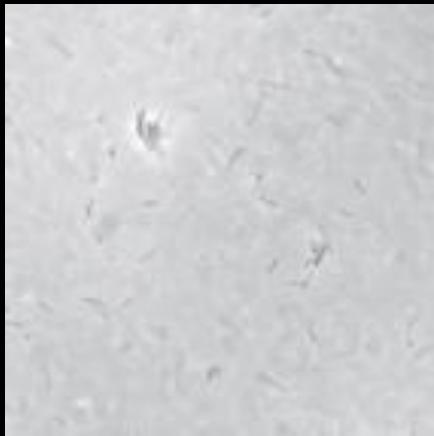
Fluorescently Labeled Secondary Antibody



Cell Surface with exposed RemA-Myc



Overexpression of RemA Causes Cell Aggregation

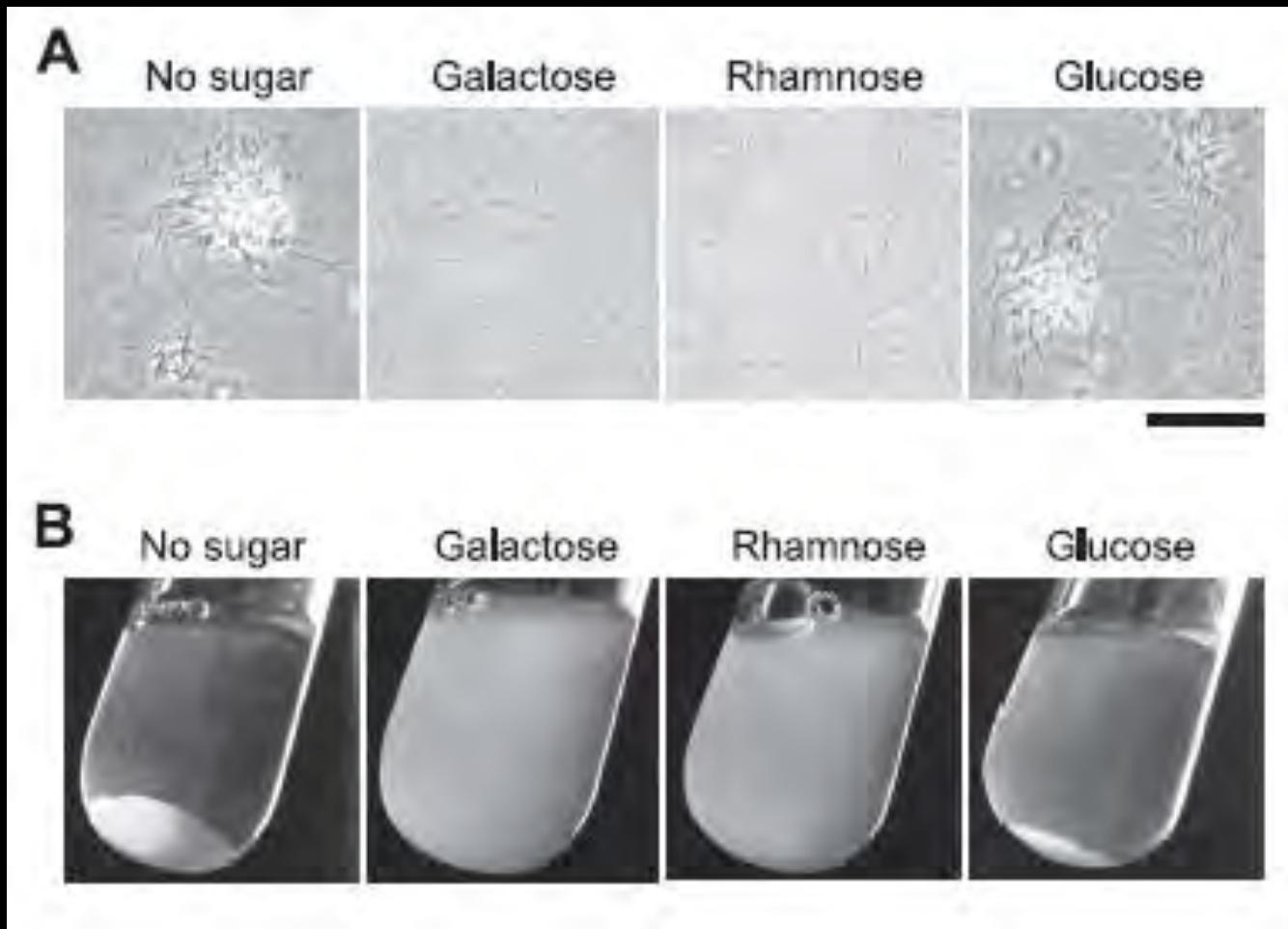


WT

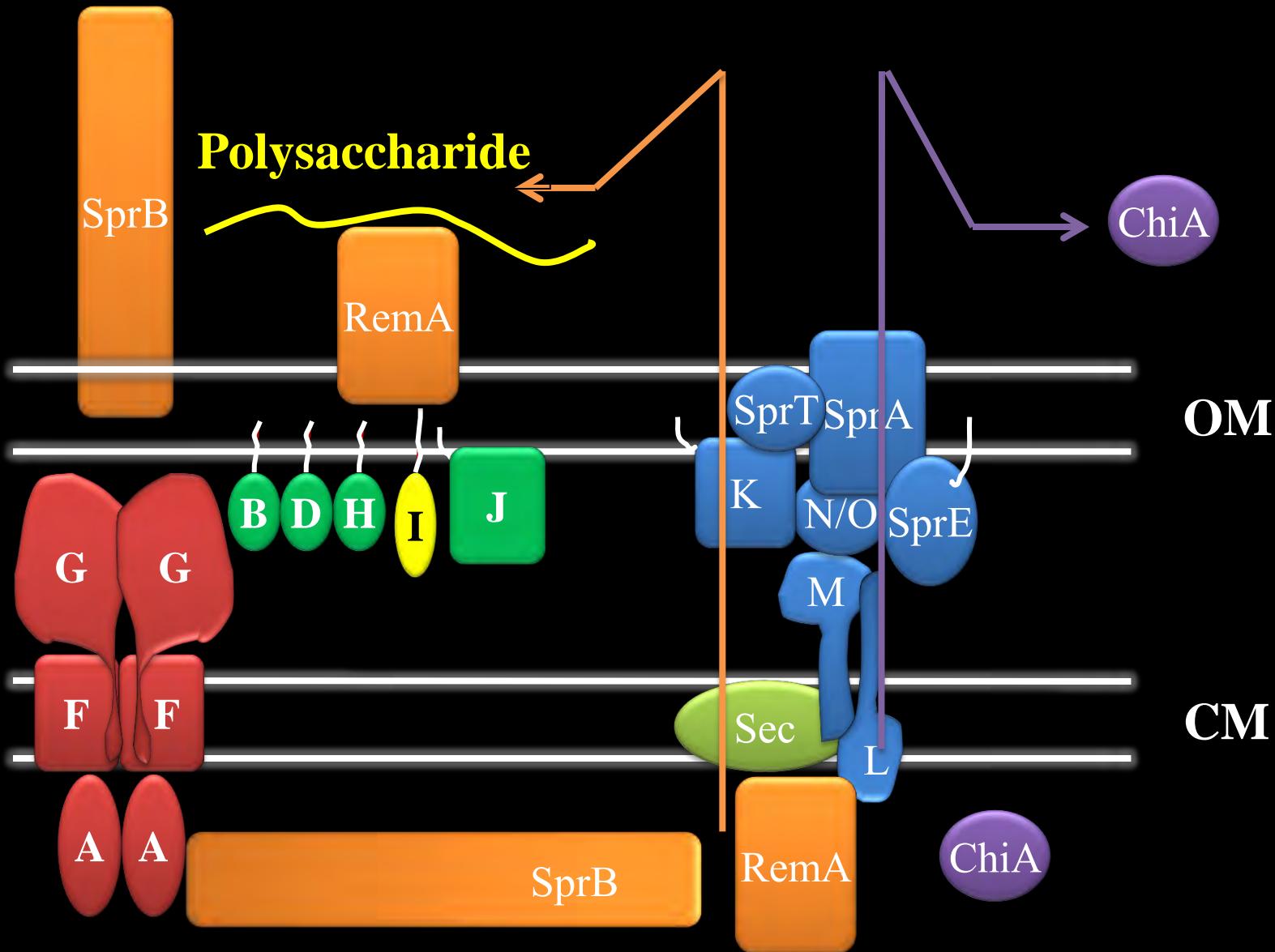


WT with RemA+++

Effect of Sugar on RemA-Mediated Cell Aggregation



‘Motility’ Proteins



Future Directions

1. Characterize redundant cell surface proteins
2. Develop assay to evaluate chemotaxis
3. Examine role of PorSS secretion in fish pathogens

Acknowledgements

McBride Lab Members

Mark McBride	Soumya Pochiraju
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Sreelekha Bollampalli	Halley Pucker
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